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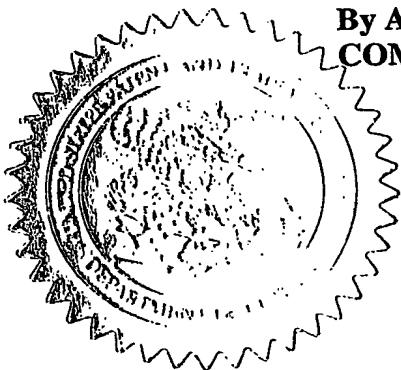
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
UTILITY PATENT APPLICATION TRANSMITTAL UNDER 37 CFR 1.53

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

J1050 U.S. PTO
J10/304234

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): Peter C.R. Emtnage, Y. Tom Tang, Zhiwei Wang, Radoje T. Drmanac

Title: METHODS OF IMMUNOTHERAPY AND DIAGNOSIS

1. Type of application

- This application is a continuation-in-part of U.S. Application Serial No. 10/128,558, filed on April 22, 2002, entitled "Novel Nucleic Acids and Polypeptides", Attorney Docket No. 812A, which in turn claims the benefit of U.S. Provisional Application Serial No. 60/339,453, filed on December 11, 2001, entitled "Novel Nucleic Acids and Polypeptides", Attorney Docket No. 812.
- Applicant hereby Requests NonPublication Under 35 U.S.C. 122(b)(2)(B)(i).
- Applicants claim small entity status (See 37 CFR 1.27)

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this Patent Application Transmittal and the documents referred to as enclosed therewith are being deposited with the United States Postal Service on November 26, 2002 in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EV211877355US



Annya Dushine

2. Application Papers Enclosed

1 Title Page
58 Pages of Specification (excluding Claims, Abstract, Drawings & Sequence Listing)
5 Page(s) of Claims
1 Page(s) of Abstract
0 Sheet(s) of Drawings Formal Informal
13 Page(s) of Sequence Listing

3. Oath or Declaration

- An executed declaration of the inventor(s) is enclosed
- Unexecuted – the undersigned attorney or agent is authorized to file this application on behalf of the applicant(s). An executed declaration will follow.

4. Additional Papers Enclosed

- Preliminary Amendment
- Information Disclosure Statement
- Declaration of Biological Deposit
- Computer readable copy of sequence listing containing nucleotide and/or amino acid sequence (1 CD-ROM)
- Statement Under 37 CFR § 1.821
- Paper Copy of Sequence Listing (13 pages)
- Assignment Document
- A bibliographic data entry sheet
- Verified translation of a non-English patent application
- Return receipt postcard
- Other _____

5. Priority Applications Under 35 USC 119

Certified copies of applications from which priority under 35 USC 119 is claimed are listed below and

- are attached.
- will follow.

6. Filing Fee Calculation (37 CFR 1.16)

A. • Utility Application

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)			SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	NO. FILED	NO. EXTRA	RATE	Fee	RATE	Fee
BASIC FEE				\$370.00		\$740.00
TOTAL	35-20	= 15	X 9 =	\$135.00	X 18 =	\$0.00
INDEP.	13-3	= 10	X 42 =	\$420.00	X 84 =	\$0.00
First Presentation of Multiple Dependent Claim			+ 140 =	140.00	+ 280 =	\$0.00
FILING FEE:			\$1065.00	OR	\$0.00	

B. Design Application (\$165.00/\$330.00) Filing Fee: \$_____

C. Plant Application (\$255.00/\$510.00) Filing Fee: \$_____

D. Other fees

Recording Assignment [Fee -- \$40.00 per assignment] \$_____

Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached [Fee -- \$130.00] \$_____

TOTAL FEES ENCLOSED \$ 1065.00

7. Method of Payments of Fees

- Enclosed check
- Charge Deposit Account No. 501169. A duplicate copy of this transmittal is enclosed

8. Deposit Account and Refund Authorization

- The Commissioner is hereby authorized to charge payment of any additional fees due or credit any overpayment to Deposit Account No. 501169. A duplicate copy of this transmittal is enclosed.

Please direct all future correspondence to Elena Quertermous at the address below.

Respectfully submitted,

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Docket No: HYS-67

METHODS OF IMMUNOTHERAPY AND DIAGNOSIS

Express Mail No. EV211877355US

METHODS OF IMMUNOTHERAPY AND DIAGNOSIS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Application Serial No.

5 10/128,558, filed on April 22, 2002, entitled "Novel Nucleic Acids and Polypeptides", Attorney Docket No. 812A, which in turn claims the benefit of U.S. Provisional Application Serial No. 60/339,453, filed on December 11, 2001, entitled "Novel Nucleic Acids and Polypeptides", Attorney Docket No. 812. These and all other U.S. Patents and Patent Applications cited herein are hereby incorporated by reference in their entirety.

10

TECHNICAL FIELD

This invention relates to compositions and methods for targeting SEQ ID NO: 2 or 4-expressing cells and their use in the therapy and diagnosis of various pathological states, including cancer, autoimmune disease, organ transplant rejection, and allergic reactions.

15

BACKGROUND

Antibody therapy for cancer involves the use of antibodies, or antibody fragments, against a tumor antigen to target antigen-expressing cells. Antibodies, or 20 antibody fragments, may have direct or indirect cytotoxic effects or may be conjugated or fused to cytotoxic moieties. Direct effects include the induction of apoptosis, the blocking of growth factor receptors, and anti-idiotype antibody formation. Indirect effects include antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-mediated cellular cytotoxicity (CMCC). When conjugated or fused to cytotoxic moieties, 25 the antibodies, or fragments thereof, provide a method of targeting the cytotoxicity towards the tumor antigen expressing cells. (Green, *et al.*, *Cancer Treatment Reviews*, 26:269-286 (2000)).

Because antibody therapy targets cells expressing a particular antigen, there is a possibility of cross-reactivity with normal cells or tissue. Although some cells, such as 30 hematopoietic cells, are readily replaced by precursors, cross-reactivity with many tissues can lead to detrimental results. Thus, considerable research has gone towards finding

tumor-specific antigens. Such antigens are found almost exclusively on tumors or are expressed at a greater level in tumor cells than the corresponding normal tissue. Tumor-specific antigens provide targets for antibody targeting of cancer, or other disease-related cells, expressing the antigen. Antibodies specific to such tumor-specific antigens can be
5 conjugated to cytotoxic compounds or can be used alone in immunotherapy.

Immunotoxins target cytotoxic compounds to induce cell death. For example, anti-CD22 antibodies conjugated to deglycosylated ricin A may be used for treatment of B cell lymphoma that has relapsed after conventional therapy (Amlot, *et al.*, *Blood* 82:2624-2633 (1993)) and has demonstrated encouraging responses in initial clinical studies.

10 Immunotherapy provides a method of harnessing the immune system to treat various pathological states, including cancer, autoimmune disease, transplant rejection, hyperproliferative conditions, and allergic reactions.

15 The immune system functions to eliminate organisms or cells that are recognized as non-self, including microorganisms, neoplasms and transplants. A cell-mediated host response to tumors includes the concept of immunologic surveillance, by which cellular mechanisms associated with cell-mediated immunity, destroy newly transformed tumor cells after recognizing tumor-associated antigens (antigens associated with tumor cells that are not apparent on normal cells). Furthermore, a humoral response to tumor-associated antigens enables destruction of tumor cells through immunological processes triggered by the binding of an antibody to the surface of a cell, such as antibody-dependent cellular cytotoxicity (ADCC) and complement mediated lysis.
20

25 Recognition of an antigen by the immune system triggers a cascade of events including cytokine production, B-cell proliferation, and subsequent antibody production. Often tumor cells have reduced capability of presenting antigen to effector cells, thus impeding the immune response against a tumor-specific antigen. In some instances, the tumor-specific antigen may not be recognized as non-self by the immune system, preventing an immune response against the tumor-specific antigen from occurring. In such instances, stimulation or manipulation of the immune system provides effective techniques of treating cancers expressing one or more tumor-specific antigens.

30 For example, Rituximab (Rituxan®) is a chimeric antibody directed against CD20, a B cell-specific surface molecule found on >95% of B-cell non-Hodgkin's

lymphoma (Press, *et al.*, *Blood* 69:584-591 (1987); Malony, *et al.*, *Blood* 90:2188-2195 (1997)). Rituximab induces ADCC and inhibits cell proliferation through apoptosis in malignant B cells *in vitro* (Maloney, *et al.*, *Blood* 88:637a (1996)). Rituximab is currently used as a therapy for advanced stage or relapsed low-grade non-Hodgkin's lymphoma, which has not responded to conventional therapy.

Active immunotherapy, whereby the host is induced to initiate an immune response against its own tumor cells can be achieved using therapeutic vaccines. One type of tumor-specific vaccine uses purified idiotype protein isolated from tumor cells, coupled to keyhole limpet hemocyanin (KLH) and mixed with adjuvant for injection into patients with low-grade follicular lymphoma (Hsu, *et al.*, *Blood* 89:3129-3135 (1997)). Another type of vaccine uses antigen-presenting cells (APCs), which present antigen to naïve T cells during the recognition and effector phases of the immune response. Dendritic cells, one type of APC, can be used in a cellular vaccine in which the dendritic cells are isolated from the patient, co-cultured with tumor antigen and then reinfused as a cellular vaccine (Hsu, *et al.*, *Nat. Med.* 2:52-58 (1996)). Immune responses can also be induced by injection of naked DNA. Plasmid DNA that expresses bicistronic mRNA encoding both the light and heavy chains of tumor idiotype proteins, such as those from B cell lymphoma, when injected into mice, are able to generate a protective, anti-tumor response (Singh, *et al.*, *Vaccine* 20:1400-1411 (2002)).

SEQ ID NO: 2 is a member of the G-protein coupled receptor(GPCR) family. GPCR's detect a wide array of extracellular signals, such as hormones, neurotransmitters, chemokines, odorants, light, and nucleotides. GPCR's convert extracellular signals to intracellular signals by activating heterotrimeric G-proteins, which in turn can interact with a broad spectrum of effector systems in order to modulate cellular signaling pathways. (Bunermann et al, *Journal of Physiology*, 517:5-23 (1999)). All GPCR's identified thus far possess a structural motif of seven membrane-spanning helices.

More specifically, SEQ ID NO: 2 appears to be most similar to purinergic receptors. Purinergic receptors respond to extracellular ATP and other extracellular nucleotides. Physiological responses to activated purinergic receptors include smooth muscle contraction, depolarization of neurons, and stimulation of the inflammatory system. Purinergic receptors have been found in almost every cell and tissue studied

(Adrian et al, Biochimica et Biophysica Acta, 1492:127-138, (2000)). As discussed below, SEQ ID NO: 2-encoding mRNA is expressed in several cancers, including lymphomas and leukemias.

SEQ ID NO: 4 possesses a predicted transmembrane domain, and SEQ ID NO: 4-
5 encoding mRNA is differentially expressed in several cancers, including lymphomas and leukemias.

Thus, there exists a need in the art to identify and develop agents, such as peptide fragments, nucleic acids, or antibodies, that provide therapeutic compositions and diagnostic methods for treating and identifying cancer, hyperproliferative disorders, auto-
10 immune diseases, organ transplant rejection, and allergy.

SUMMARY OF THE INVENTION

The invention provides therapeutic and diagnostic methods of targeting cells expressing SEQ ID NO: 2 or 4 by using targeting elements such as SEQ ID NO: 2 or 4-
15 polypeptides, nucleic acids encoding SEQ ID NO: 2 or 4 protein, and anti-SEQ ID NO: 2 or 4 antibodies, including fragments or other modifications thereof. SEQ ID NO: 2 or 4 is highly expressed in certain hematopoietic-based cancer cells relative to its expression in healthy cells. Thus, targeting of cells that express SEQ ID NO: 2 or 4 will have a minimal effect on healthy tissues while destroying or inhibiting the growth of the
20 hematopoietic-based cancer cells. Similarly, non-hematopoietic type tumors (solid tumors) can be targeted if they bear the SEQ ID NO: 2 or 4 antigen. For example, inhibition of growth and /or destruction of SEQ ID NO: 2 or 4-expressing cancer cells results from targeting such cells with anti-SEQ ID NO: 2 or 4 antibodies. One embodiment of the invention is a method of destroying SEQ ID NO: 2 or 4-expressing
25 cells by conjugating anti-SEQ ID NO: 2 or 4 antibodies with cytoidal materials such as radioisotopes or other cytotoxic compounds.

The present invention provides a variety of targeting elements and compositions. One such embodiment is a composition comprising an anti-SEQ ID NO: 2 or 4 antibody preparation. Exemplary antibodies include a single anti-SEQ ID NO: 2 or 4 antibody, a
30 combination of two or more anti-SEQ ID NO: 2 or 4 antibodies, a combination of a anti-SEQ ID NO: 2 or 4 antibody with a non-SEQ ID NO: 2 or 4 antibody, a combination of

anti-SEQ ID NO: 2 or 4 antibody and a therapeutic agent, a combination of an anti-SEQ ID NO: 2 or 4 antibody and a cytocidal agent, a bispecific anti-SEQ ID NO: 2 or 4 antibody, Fab SEQ ID NO: 2 or 4 antibodies or fragments thereof, including any fragment of an antibody that retains one or more CDRs that recognize SEQ ID NO: 2 or 5 4, humanized anti-SEQ ID NO: 2 or 4 antibodies that retain all or a portion of a CDR that recognizes SEQ ID NO: 2 or 4, anti-SEQ ID NO: 2 or 4 conjugates, and anti-SEQ ID NO: 2 or 4 antibody fusion proteins.

Another targeting embodiment of the invention is a vaccine comprising a SEQ ID NO: 2 or 4 polypeptide, or a fragment or variant thereof and optionally comprising a 10 suitable adjuvant.

Yet another targeting embodiment is a composition comprising a nucleic acid encoding SEQ ID NO: 2 or 4, or a fragment or variant thereof, optionally within a recombinant vector. A further targeting embodiment of the present invention is a composition comprising an antigen-presenting cell transformed with a nucleic acid 15 encoding SEQ ID NO: 2 or 4, or a fragment or variant thereof, optionally within a recombinant vector. The present invention further provides a method of targeting SEQ ID NO: 2 or 4-expressing cells, which comprises administering a targeting element or composition in an amount effective to target SEQ ID NO: 2 or 4-expressing cells. Any one of the targeting elements or compositions described herein may be used in such 20 methods, including an anti-SEQ ID NO: 2 or 4 antibody preparation, a vaccine comprising a SEQ ID NO: 2 or 4 polypeptide, or a fragment or variant thereof or a composition of a nucleic acid encoding SEQ ID NO: 2 or 4, or a fragment or variant thereof, optionally within a recombinant vector or a composition of an antigen-presenting cell transformed with a nucleic acid encoding SEQ ID NO: 2 or 4, or fragment or variant 25 thereof, optionally within a recombinant vector.

The invention also provides a method of inhibiting the growth of hematopoietic-based, SEQ ID NO: 2 or 4-expressing cancer cells, which comprises administering a targeting element or a targeting composition in an amount effective to inhibit the growth of said hematopoietic-based cancer cells. Any one of the targeting elements or 30 compositions described herein may be used in such methods, including an anti-SEQ ID NO: 2 or 4 antibody preparation, a vaccine comprising a SEQ ID NO: 2 or 4 polypeptide,

fragment, or variant thereof, composition of a nucleic acid encoding SEQ ID NO: 2 or 4, or fragment or variant thereof, optionally within a recombinant vector, or a composition of an antigen-presenting cell transformed with a nucleic acid encoding SEQ ID NO: 2 or 4, or fragment or variant thereof, optionally within a recombinant vector.

5

The present invention further provides a method of treating disorders associated with the proliferation of SEQ ID NO: 2 or 4 - expressing cells in a subject in need thereof, comprising the step of administering a targeting element or targeting composition in a therapeutically effective amount to treat disorders associated with SEQ ID NO: 2 or 4- expressing cells. Any one of the targeting elements or compositions described herein may be used in such methods, including an anti-SEQ ID NO: 2 or 4 antibody preparation, a vaccine comprising a SEQ ID NO: 2 or 4 polypeptide, fragment, or variant thereof, a composition of a nucleic acid encoding SEQ ID NO: 2 or 4, or fragment or variant thereof, optionally within a recombinant vector, or a composition of an antigen-presenting cell comprising a nucleic acid encoding SEQ ID NO: 2 or 4, or fragment or variant thereof, optionally within a recombinant vector. Examples of disorders associated with the proliferation of SEQ ID NO: 2 or 4- expressing cells include cancers, such as non-Hodgkin's B-cell lymphomas, B-cell leukemias, chronic lymphocytic leukemia, multiple myeloma, acute and chronic myeloid leukemia; myelodysplastic syndromes; T cell lymphomas, X-linked lymphoproliferative disorders; Epstein Barr Virus-related conditions such as mononucleosis; autoimmune disorders; and allergy. Non-hematopoietic tumors that bear the SEQ ID NO: 2 or 4 antigen can also be targeted. The invention further provides a method of modulating the immune system by either suppression or stimulation of growth factors and cytokines, by administering the targeting elements or compositions of the invention. The invention also provides a method of modulating the immune system through activation of immune cells (such as natural killer cells, T cells, B cells and myeloid cells), through the suppression of activation, or by stimulating or suppressing proliferation of these cells by SEQ ID NO: 2 or 4 peptide fragments or SEQ ID NO: 2 or 4 antibodies.

30 The present invention thereby provides a method of treating immune-related disorders by suppressing the immune system in a subject in need thereof, by

administering the targeting elements or compositions of the invention. Such immune-related disorders include but are not limited to autoimmune disease and organ transplant rejection.

The present invention also provides a method of diagnosing disorders associated with SEQ ID NO: 2 or 4 – expressing cells comprising the step of measuring the expression patterns of SEQ ID NO: 2 or 4 protein and/or mRNA. Yet another embodiment of a method of diagnosing disorders associated with SEQ ID NO: 2 or 4-expressing cells comprising the step of detecting SEQ ID NO: 2 or 4 expression using anti-SEQ ID NO: 2 or 4 antibodies. Such methods of diagnosis include compositions, 10 kits and other approaches for determining whether a patient is a candidate for SEQ ID NO: 2 or 4-based immunotherapy.

The present invention also provides a method of enhancing the effects of therapeutic agents and adjunctive agents used to treat and manage disorders associated with SEQ ID NO: 2 or 4-expressing cells, by administering SEQ ID NO: 2 or 4 15 preparations with therapeutic and adjuvant agents commonly used to treat such disorders.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods of targeting cells that express SEQ ID NO: 2 or 4 using targeting elements, such as SEQ ID NO: 2 or 4 polypeptides, nucleic acids encoding SEQ ID NO: 2 or 4, anti-SEQ ID NO: 2 or 4 antibodies, including 20 fragments or other modifications of any of these elements.

The present invention provides a novel approach for diagnosing and treating diseases and disorders associated with SEQ ID NO: 2 or 4 -expressing cells. The method comprises administering an effective dose of targeting preparations such as vaccines, 25 antigen presenting cells, or pharmaceutical compositions comprising the targeting elements, SEQ ID NO: 2 or 4 polypeptides, nucleic acids encoding SEQ ID NO: 2 or 4, anti-SEQ ID NO: 2 or 4 antibodies, described below. Targeting of SEQ ID NO: 2 or 4 on the cell membranes of SEQ ID NO: 2 or 4-expressing cells is expected to inhibit the growth of or destroy such cells. An effective dose will be the amount of such targeting 30 SEQ ID NO: 2 or 4 preparations necessary to target the SEQ ID NO: 2 or 4 on the cell

membrane and inhibit the growth of or destroy the SEQ ID NO: 2 or 4-expressing cells and/or metastasis.

A further embodiment of the present invention is to enhance the effects of therapeutic agents and adjunctive agents used to treat and manage disorders associated with SEQ ID NO: 2 or 4 – expressing cells, by administering SEQ ID NO: 2 or 4 preparations with therapeutic and adjuvant agents commonly used to treat such disorders. Chemotherapeutic agents useful in treating neoplastic disease and antiproliferative agents and drugs used for immunosuppression include alkylating agents, such as nitrogen mustards, alkyl sulfonates, nitrosoureas, triazenes; antimetabolites, such as folic acid analogs, pyrimidine analogs, and purine analogs; natural products, such as vinca alkaloids, epipodophyllotoxins, antibiotics, and enzymes; miscellaneous agents such as polatinium coordination complexes, substituted urea, methyl hydrazine derivatives, and adrenocortical suppressant; and hormones and antagonists, such as adrenocorticosteroids, progestins, estrogens, androgens, and anti-estrogens (Calebresi and Parks, pp. 1240-1306 in, Eds. A.G Goodman, L.S. Goodman, T.W. Rall, and F. Murad, *The Pharmacological Basis of Therapeutics*, Seventh Edition, MacMillan Publishing Company, New York, (1985)).

Adjunctive therapy used in the management of such disorders includes, for example, radiosensitizing agents, coupling of antigen with heterologous proteins, such as globulin or beta-galactosidase, or inclusion of an adjuvant during immunization.

High doses may be required for some therapeutic agents to achieve levels to effectuate the target response, but may often be associated with a greater frequency of dose-related adverse effects. Thus, combined use of the immunotherapeutic methods of the present invention with agents commonly used to treat SEQ ID NO: 2 or 4 protein-related disorders allows the use of relatively lower doses of such agents resulting in a lower frequency of adverse side effects associated with long-term administration of the conventional therapeutic agents. Thus another indication for the immunotherapeutic methods of this invention is to reduce adverse side effects associated with conventional therapy of disorders associated with SEQ ID NO: 2 or 4-expressing cells.

30

DEFINITIONS

The term "fragment" of a nucleic acid refer to a sequence of nucleotide residues which are at least about 5 nucleotides, more preferably at least about 7 nucleotides, more preferably at least about 9 nucleotides, more preferably at least about 11 nucleotides and most preferably at least about 17 nucleotides. The fragment is preferably less than about 5 500 nucleotides, preferably less than about 200 nucleotides, more preferably less than about 100 nucleotides, more preferably less than about 50 nucleotides and most preferably less than 30 nucleotides. Preferably the fragments can be used in polymerase chain reaction (PCR), various hybridization procedures or microarray procedures to identify or amplify identical or related parts of mRNA or DNA molecules. A fragment or 10 segment may uniquely identify each polynucleotide sequence of the present invention. Preferably the fragment comprises a sequence substantially similar to a portion of SEQ ID NO: SEQ ID NO: 2 or 4. A polypeptide "fragment" is a stretch of amino acid residues of at least about 5 amino acids, preferably at least about 7 amino acids, more preferably at least about 9 amino acids and most preferably at least about 17 or more 15 amino acids. The peptide preferably is not greater than about 200 amino acids, more preferably less than 150 amino acids and most preferably less than 100 amino acids. Preferably the peptide is from about 5 to about 200 amino acids. To be active, any polypeptide must have sufficient length to display biological and/or immunological activity. The term "immunogenic" refers to the capability of the natural, recombinant or 20 synthetic SEQ ID NO: 2 or 4-like peptide, or any peptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term "variant"(or "analog") refers to any polypeptide differing from naturally occurring polypeptides by amino acid insertions, deletions, and substitutions, created using, e.g., recombinant DNA techniques. Guidance in determining which amino acid 25 residues may be replaced, added or deleted without abolishing activities of interest, may be found by comparing the sequence of the particular polypeptide with that of homologous peptides and minimizing the number of amino acid sequence changes made in regions of high homology (conserved regions) or by replacing amino acids with consensus sequence.

Alternatively, recombinant variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code.

Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations in the polynucleotide sequence may be reflected in the polypeptide or domains of other peptides added to the polypeptide to modify the properties of any part of the polypeptide, to change characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate.

IMMUNOTARGETING OF SEQ ID NO: 2 OR 4

The amino acid sequence of an exemplary polypeptide and the nucleic acid sequence of the cDNA encoding the polypeptide are provided in SEQ ID NOS: 1 and 2.

- 5 The data described herein in Examples 1 and 2 show SEQ ID NO: 2 is expressed in certain hematopoietic-based cancers, while most non-hematopoietic, healthy cells fail to express SEQ ID NO: 2. Thus, targeting SEQ ID NO: 2 will have a minimal effect on healthy tissue while destroying or inhibiting the growth of the hematopoietic-based cancer cells
- 10 The amino acid sequence of another exemplary polypeptide and the nucleic acid sequence of the cDNA encoding the polypeptide are provided in SEQ ID NOS: 3 and 4. The data described herein in Examples 1 and 2 show SEQ ID NO: 4 is expressed in certain hematopoietic-based cancers, while most non-hematopoietic, healthy cells fail to express SEQ ID NO: 4. Thus, targeting SEQ ID NO: 4 will have a minimal effect on healthy tissue while destroying or inhibiting the growth of the hematopoietic-based cancer cells

A. TARGETING USING SEQ ID NO: 2 OR 4 VACCINES

One embodiment the present invention provides a vaccine comprising a SEQ ID NO: 2 or 4 polypeptide to stimulate the immune system against SEQ ID NO: 2 or 4, thus targeting SEQ ID NO: 2 or 4-expressing cells. Use of a tumor antigen in a vaccine for generating cellular and humoral immunity for the purpose of anti-cancer therapy is well known in the art. For example, one type of tumor-specific vaccine uses purified idiotype protein isolated from tumor cells, coupled to keyhole limpet hemocyanin (KLH) and

- 20

mixed with adjuvant for injection into patients with low-grade follicular lymphoma (Hsu, *et al.*, *Blood* 89: 3129-3135 (1997)). U.S. Patent No. 6,312,718 describes methods for inducing immune responses against malignant B cells, in particular lymphoma, chronic lymphocytic leukemia, and multiple myeloma. The methods described therein utilize vaccines that include liposomes having (1) at least one B-cell malignancy-associated antigen, (2) IL-2 alone, or in combination with at least one other cytokine or chemokine, and (3) at least one lipid molecule. Methods of vaccinating against SEQ ID NO: 2 or 4 typically employ a SEQ ID NO: 2 or 4 polypeptide, including fragments, analogs and variants.

As another example, dendritic cells, one type of antigen-presenting cell, can be used in a cellular vaccine in which the dendritic cells are isolated from the patient, co-cultured with tumor antigen and then reinfused as a cellular vaccine (Hsu, *et al.*, *Nat. Med.* 2:52-58 (1996)).

15 B. **TARGETING USING NUCLEIC ACIDS ENCODING SEQ ID NO: 2 OR 4**

1 1. DIRECT DELIVERY OF NUCLEIC ACIDS

However, in some embodiments, a nucleic acid encoding SEQ ID NO: 2 or 4, or encoding a fragment, analog or variant thereof, within a recombinant vector is utilized. Such methods are known in the art. For example, immune responses can be induced by injection of naked DNA. Plasmid DNA that expresses bicistronic mRNA encoding both the light and heavy chains of tumor idiotype proteins, such as those from B cell lymphoma, when injected into mice, are able to generate a protective, anti-tumor response (Singh, *et al.*, *Vaccine* 20:1400-1411 (2002)). SEQ ID NO: 2 or 4 viral vectors are particularly useful for delivering SEQ ID NO: 2 or 4-encoding nucleic acids to cells. Examples of vectors include those derived from influenza, adenovirus, vaccinia, herpes simplex virus, fowlpox, vesicular stomatitis virus, canarypox, poliovirus, adeno-associated virus, and lentivirus and sindbus virus. Of course, non-viral vectors, such as liposomes or even naked DNA, are also useful for delivering SEQ ID NO: 2 or 4-encoding nucleic acids to cells.

Combining this type of therapy with other types of therapeutic agents or treatments such as chemotherapy or radiation is also contemplated.

2. NUCLEIC ACIDS EXPRESSED ENCODING SEQ ID NO: 2 OR 4 IN CELLS

In some embodiments, a vector comprising a nucleic acid encoding the SEQ ID NO: 2 or 4 polypeptide (including a fragment, analog or variant) is introduced into a cell, such as a dendritic cell or a macrophage. When expressed in an antigen-presenting cell, SEQ ID NO: 2 or 4 antigens are presented to T cells eliciting an immune response against SEQ ID NO: 2 or 4. Such methods are also known in the art. Methods of introducing tumor antigens into antigen presenting cells and vectors useful therefor are described in U.S. Patent No. 6,300,090. The vector encoding SEQ ID NO: 2 or 4 may be introduced into the antigen presenting cells *in vivo*. Alternatively, antigen-presenting cells are loaded with SEQ ID NO: 2 or 4 or a nucleic acid encoding SEQ ID NO: 2 or 4 *ex vivo* and then introduced into a patient to elicit an immune response against SEQ ID NO: 2 or 4. In another alternative, the cells presenting SEQ ID NO: 2 or 4 antigen are used to stimulate the expansion of anti-SEQ ID NO: 2 or 4 cytotoxic T lymphocytes (CTL) *ex vivo* followed by introduction of the stimulated CTL into a patient. (U.S. Patent No. 6,306,388)

Combining this type of therapy with other types of therapeutic agents or treatments such as chemotherapy or radiation is also contemplated.

20 C. ANTI-SEQ ID NO: 2 OR 4 ANTIBODIES

Alternatively, immunotargeting involves the administration of components of the immune system, such as antibodies, antibody fragments, or primed cells of the immune system against the target. Methods of immunotargeting cancer cells using antibodies or antibody fragments are well known in the art. U.S. Patent No. 6,306,393 describes the use of anti-CD22 antibodies in the immunotherapy of B-cell malignancies, and U.S. Patent No. 6,329,503 describes immunotargeting of cells that express serpentine transmembrane antigens.

SEQ ID NO: 2 or 4 antibodies (including humanized or human monoclonal antibodies or fragments or other modifications thereof, optionally conjugated to cytotoxic agents) may be introduced into a patient such that the antibody binds to SEQ ID NO: 2 or 4 expressed by cancer cells and mediates the destruction of the cells and the tumor and/or

inhibits the growth of the cells or the tumor. Without intending to limit the disclosure, mechanisms by which such antibodies can exert a therapeutic effect may include complement-mediated cytosis, antibody-dependent cellular cytotoxicity (ADCC), modulating the physiologic function of SEQ ID NO: 2 or 4, inhibiting binding or signal transduction pathways, modulating tumor cell differentiation, altering tumor angiogenesis factor profiles, modulating the secretion of immune stimulating or tumor suppressing cytokines and growth factors, modulating cellular adhesion, and/or by inducing apoptosis. SEQ ID NO: 2 or 4 antibodies conjugated to toxic or therapeutic agents, such as radioligands or cytosolic toxins, may also be used therapeutically to deliver the toxic or therapeutic agent directly to SEQ ID NO: 2 or 4-bearing tumor cells.

SEQ ID NO: 2 or 4 antibodies may be used to suppress the immune system in patients receiving organ transplants or in patients with autoimmune diseases such as arthritis. Healthy immune cells would be targeted by these antibodies leading their death and clearance from the system, thus suppressing the immune system.

SEQ ID NO: 2 or 4 antibodies may be used as antibody therapy for solid tumors which express this action. Cancer immunotherapy using antibodies provides a novel approach to treating cancers associated with cells that specifically express SEQ ID NO: 2 or 4. Cancer immunotherapy using antibodies has been previously described for other types of cancer, including but not limited to colon cancer (Arlen, *et al.*, *Crit. Rev. Immunol.* 18:133-138 (1998)), multiple myeloma (Ozaki, *et al.*, *Blood* 90:3179-3186 (1997); Tsunenari, *et al.*, *Blood* 90:2437-2444 (1997)), gastric cancer (Kasprzyk, *et al.*, *Cancer Res.* 52:2771-2776 (1992)), B-cell lymphoma (Funakoshi, *et al.*, *J. Immunother. Emphasis Tumor Immunol.* 19:93-101 (1996)), leukemia (Zhong, *et al.*, *Leuk. Res.* 20:581-589 (1996)), colorectal cancer (Moun, *et al.*, *Cancer Res.* 54:6160-6166 (1994); Velders, *et al.*, *Cancer Res.* 55:4398-4403 (1995)), and breast cancer (Shepard, *et al.*, *J. Clin. Immunol.* 11:117-127 (1991)).

Although anti-SEQ ID NO: 2 or 4 antibody therapy may be useful for all stages of the foregoing cancers, antibody therapy may be particularly appropriate in advanced or metastatic cancers. Combining the antibody therapy method with a chemotherapeutic, radiation or surgical regimen may be preferred in patients that have not received chemotherapeutic treatment, whereas treatment with the antibody therapy may be

indicated for patients who have received one or more chemotherapies. Additionally, antibody therapy can also enable the use of reduced dosages of concomitant chemotherapy, particularly in patients that do not tolerate the toxicity of the chemotherapeutic agent very well. Furthermore, treatment of cancer patients with anti-

5 SEQ ID NO: 2 or 4 antibody with tumors resistant to chemotherapeutic agents might induce sensitivity and responsiveness to these agents in combination.

Prior to anti-SEQ ID NO: 2 or 4 immunotargeting, a patient may be evaluated for the presence and level of SEQ ID NO: 2 or 4 expression by the cancer cells, preferably using immunohistochemical assessments of tumor tissue, quantitative SEQ ID NO: 2 or 4 imaging, quantitative RT-PCR, or other techniques capable of reliably indicating the presence and degree of SEQ ID NO: 2 or 4 expression. For example, a blood or biopsy sample may be evaluated by immunohistochemical methods to determine the presence of SEQ ID NO: 2 or 4-expressing cells or to determine the extent of SEQ ID NO: 2 or 4 expression on the surface of the cells within the sample. Methods for 10 immunohistochemical analysis of tumor tissues or released fragments of SEQ ID NO: 2 or 4 in the serum are well known in the art.

Anti-SEQ ID NO: 2 or 4 antibodies useful in treating cancers include those, which are capable of initiating a potent immune response against the tumor and those, which are capable of direct cytotoxicity. In this regard, anti-SEQ ID NO: 2 or 4 mAbs may elicit 20 tumor cell lysis by either complement-mediated or ADCC mechanisms, both of which require an intact Fc portion of the immunoglobulin molecule for interaction with effector cell Fc receptor sites or complement proteins. In addition, anti-SEQ ID NO: 2 or 4 antibodies that exert a direct biological effect on tumor growth are useful in the practice of the invention. Potential mechanisms by which such directly cytotoxic antibodies may 25 act include inhibition of cell growth, modulation of cellular differentiation, modulation of tumor angiogenesis factor profiles, and the induction of apoptosis. The mechanism by which a particular anti-SEQ ID NO: 2 or 4 antibody exerts an anti-tumor effect may be evaluated using any number of *in vitro* assays designed to determine ADCC, ADMMC, complement-mediated cell lysis, and so forth, as is generally known in the art.

30 The anti-tumor activity of a particular anti-SEQ ID NO: 2 or 4 antibody, or combination of anti-SEQ ID NO: 2 or 4 antibody, may be evaluated *in vivo* using a

suitable animal model. For example, xenogenic lymphoma cancer models wherein human lymphoma cells are introduced into immune compromised animals, such as nude or SCID mice. Efficacy may be predicted using assays, which measure inhibition of tumor formation, tumor regression or metastasis, and the like.

5 It should be noted that the use of murine or other non-human monoclonal antibodies, human/mouse chimeric mAbs may induce moderate to strong immune responses in some patients. In the most severe cases, such an immune response may lead to the extensive formation of immune complexes, which, potentially, can cause renal failure. Accordingly, preferred monoclonal antibodies used in the practice of the
10 therapeutic methods of the invention are those which are either fully human or humanized and which bind specifically to the target SEQ ID NO: 2 or 4 antigen with high affinity but exhibit low or no antigenicity in the patient.

The method of the invention contemplates the administration of single anti-SEQ ID NO: 2 or 4 monoclonal antibodies (mAbs) as well as combinations, or "cocktails", of
15 different mAbs. Two or more monoclonal antibodies that bind to SEQ ID NO: 2 or 4 may provide an improved effect compared to a single antibody. Alternatively, a combination of an anti-SEQ ID NO: 2 or 4 antibody with an antibody that binds a different antigen may provide an improved effect compared to a single antibody. Such mAb cocktails may have certain advantages inasmuch as they contain mAbs, which
20 exploit different effector mechanisms or combine directly cytotoxic mAbs with mAbs that rely on immune effector functionality. Such mAbs in combination may exhibit synergistic therapeutic effects. In addition, the administration of anti-SEQ ID NO: 2 or 4 mAbs may be combined with other therapeutic agents, including but not limited to various chemotherapeutic agents, androgen-blockers, and immune modulators (e.g., IL-2,
25 GM-CSF). The anti-SEQ ID NO: 2 or 4 mAbs may be administered in their "naked" or unconjugated form, or may have therapeutic agents conjugated to them. Additionally, bispecific antibodies may be used. Such an antibody would have one antigenic binding domain specific for SEQ ID NO: 2 or 4 and the other antigenic binding domain specific for another antigen (such as CD20 for example). Finally, Fab SEQ ID NO: 2 or 4
30 antibodies or fragments of these antibodies (including fragments conjugated to other protein sequences or toxins) may also be used as therapeutic agents.

(1) ANTI-SEQ ID NO: 2 OR 4 ANTIBODIES

Antibodies that specifically bind SEQ ID NO: 2 or 4 are useful in compositions and methods for immunotargeting cells expressing SEQ ID NO: 2 or 4 and for diagnosing 5 a disease or disorder wherein cells involved in the disorder express SEQ ID NO: 2 or 4. Such antibodies include monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, bifunctional/bispecific antibodies, humanized antibodies, human antibodies, and complementary determining region (CDR)-grafted antibodies, including compounds that include CDR and/or antigen-binding sequences, which specifically 10 recognize SEQ ID NO: 2 or 4. Antibody fragments, including Fab, Fab', F(ab')₂, and F_v, are also useful.

The term "specific for" indicates that the variable regions of the antibodies recognize and bind SEQ ID NO: 2 or 4 exclusively (*i.e.*, able to distinguish SEQ ID NO: 2 or 4 from other similar polypeptides despite sequence identity, homology, or similarity 15 found in the family of polypeptides), but may also interact with other proteins (for example, *S. aureus* protein A or other antibodies in ELISA techniques) through interactions with sequences outside the variable region of the antibodies, and in particular, in the constant region of the molecule. Screening assays in which one can determine binding specificity of an anti-SEQ ID NO: 2 or 4 antibody are well known and 20 routinely practiced in the art. (*Chapter 6, Antibodies A Laboratory Manual*, Eds. Harlow, *et al.*, Cold Spring Harbor Laboratory; Cold Spring Harbor, NY (1988)).

SEQ ID NO: 2 or 4 polypeptides can be used to immunize animals to obtain polyclonal and monoclonal antibodies that specifically react with SEQ ID NO: 2 or 4. Such antibodies can be obtained using either the entire protein or fragments thereof as an 25 immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides have been previously described (Merrifield, *J. Amer. Chem. Soc.* 85, 2149-2154 (1963); Krstenansky, *et al.*, *FEBS Lett.* 211: 10 (1987)). Techniques for preparing polyclonal and monoclonal antibodies as well 30 as hybridomas capable of producing the desired antibody have also been previously disclosed (Campbell, *Monoclonal Antibodies Technology: Laboratory Techniques in*

Biochemistry and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth, *et al.*, *J. Immunol.* 35:1-21 (1990); Kohler and Milstein, *Nature* 256:495-497 (1975)), the trioma technique, the human B-cell hybridoma technique (Kozbor, *et al.*, *Immunology Today* 4:72 (1983); Cole, *et al.*, in, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)).

Any animal capable of producing antibodies can be immunized with a SEQ ID NO: 2 or 4 peptide or polypeptide. Methods for immunization include subcutaneous or intraperitoneal injection of the polypeptide. The amount of the SEQ ID NO: 2 or 4 peptide or polypeptide used for immunization depends on the animal that is immunized, antigenicity of the peptide and the site of injection. The SEQ ID NO: 2 or 4 peptide or polypeptide used as an immunogen may be modified or administered in an adjuvant in order to increase the protein's antigenicity. Methods of increasing the antigenicity of a protein are well known in the art and include, but are not limited to, coupling the antigen with a heterologous protein (such as globulin or β -galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells. Any one of a number of methods well known in the art can be used to identify the hybridoma cell that produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, Western blot analysis, or radioimmunoassay (Lutz, *et al.*, *Exp. Cell Res.* 175:109-124 (1988)). Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, A.M., *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1984)). Techniques described for the production of single chain antibodies can be adapted to produce single chain antibodies to SEQ ID NO: 2 or 4 (U.S. Patent 4,946,778).

For polyclonal antibodies, antibody-containing antiserum is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures.

Because antibodies from rodents tend to elicit strong immune responses against the antibodies when administered to a human, such antibodies may have limited effectiveness in therapeutic methods of the invention. Methods of producing antibodies that do not produce a strong immune response against the administered antibodies are well known in the art. For example, the anti-SEQ ID NO: 2 or 4 antibody can be a nonhuman primate antibody. Methods of making such antibodies in baboons are disclosed in WO 91/11465 and Losman *et al.*, *Int. J. Cancer* 46:310-314 (1990). In one embodiment, the anti-SEQ ID NO: 2 or 4 antibody is a humanized monoclonal antibody. Methods of producing humanized antibodies have been previously described. (U.S. Patent Nos. 5,997,867 and 5,985,279, Jones *et al.*, *Nature* 321:522 (1986); Riechmann *et al.*, *Nature* 332:323(1988); Verhoeven *et al.*, *Science* 239:1534-1536 (1988); Carter *et al.*, *Proc. Nat'l Acad. Sci. USA* 89:4285-4289 (1992); Sandhu, *Crit. Rev. Biotech.* 12:437-462 (1992); and Singer, *et al.*, *J. Immun.* 150:2844-2857 (1993)). In another embodiment, the anti-SEQ ID NO: 2 or 4 antibody is a human monoclonal antibody. Humanized antibodies are produced by transgenic mice that have been engineered to produce human antibodies. Hybridomas derived from such mice will secrete large amounts of human monoclonal antibodies. Methods for obtaining human antibodies from transgenic mice are described in Green , *et al.*, *Nature Genet.* 7:13-21(1994), Lonberg, *et al.*, *Nature* 368:856 (1994), and Taylor, *et al.*, *Int. Immun.* 6:579 (1994).

The present invention also includes the use of anti-SEQ ID NO: 2 or 4 antibody fragments. Antibody fragments can be prepared by proteolytic hydrolysis of an antibody or by expression in *E. coli* of the DNA coding for the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulphydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab fragments and an Fc fragment directly. These methods have been previously described (U.S. Patent Nos. 4,036,945 and 4,331,647, Nisonoff, *et al.*, *Arch Biochem. Biophys.* 89:230 (1960); Porter, *Biochem. J.* 73:119 (1959), Edelman, *et al.*, *Meth. Enzymol.* 1:422 (1967)). Other

methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody. For example, Fv fragments comprise an association 5 of V_H and V_L chains, which can be noncovalent (Inbar *et al.*, *Proc. Nat'l Acad. Sci. USA* 69:2659 (1972)). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde.

In one embodiment, the Fv fragments comprise V_H and V_L chains that are connected by a peptide linker. These single-chain antigen binding proteins (sFv) are 10 prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains which are connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell, such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs have been previously 15 described (U.S. Patent No. 4,946,778, Whitlow, *et al.*, *Methods: A Companion to Methods in Enzymology* 2:97 (1991), Bird, *et al.*, *Science* 242:423 (1988), Pack, *et al.*, *Bio/Technology* 11:1271 (1993)).

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition 20 units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (Larrick, *et al.*, *Methods: A Companion to Methods in Enzymology* 2:106 (1991); Courtenay-Luck, pp. 166-179 in, *Monoclonal Antibodies Production, Engineering and Clinical Applications*, 25 Eds. Ritter *et al.*, Cambridge University Press (1995); Ward, *et al.*, pp. 137-185 in, *Monoclonal Antibodies Principles and Applications*, Eds. Birch *et al.*, Wiley-Liss, Inc. (1995)).

The present invention further provides the above-described antibodies in detectably labeled form. Antibodies can be detectably labeled through the use of 30 radioisotopes, affinity labels (such as biotin, avidin, etc.), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, etc.) fluorescent labels (such as FITC or

rhodamine, etc.), paramagnetic atoms, etc. Procedures for accomplishing such labeling have been previously disclosed (Sternberger, *et al.*, *J. Histochem. Cytochem.* 18:315 (1970); Bayer, *et al.*, *Meth. Enzym.* 62:308 (1979); Engval, *et al.*, *Immunol.* 109:129 (1972); Goding, *J. Immunol. Meth.* 13:215 (1976)).

5 The labeled antibodies can be used for *in vitro*, *in vivo*, and *in situ* assays to identify cells or tissues in which SEQ ID NO: 2 or 4 is expressed. Furthermore, the labeled antibodies can be used to identify the presence of secreted SEQ ID NO: 2 or 4 in a biological sample, such as a blood, urine, saliva samples.

10 (2) **ANTI-SEQ ID NO: 2 OR 4 ANTIBODY CONJUGATES**

The present invention contemplates the use of "naked" anti-SEQ ID NO: 2 or 4 antibodies, as well as the use of immunoconjugates. Immunoconjugates can be prepared by indirectly conjugating a therapeutic agent such as a cytotoxic agent to an antibody component. Toxic moieties include, for example, plant toxins, such as abrin, ricin, modeccin, viscumin, pokeweed anti-viral protein, saporin, gelonin, momordin, trichosanthin, barley toxin; bacterial toxins, such as *Diphtheria* toxin, *Pseudomonas* endotoxin and exotoxin, *Staphylococcal* enterotoxin A; fungal toxins, such as α -sarcin, restrictocin; cytotoxic RNases, such as extracellular pancreatic RNases; DNase I (Pastan, *et al.*, *Cell* 47:641 (1986); Goldenberg, *Cancer Journal for Clinicians* 44:43 (1994)), calicheamicin, and radioisotopes, such as ^{32}P , ^{67}Cu , ^{77}As , ^{105}Rh , ^{109}Pd , ^{111}Ag , ^{121}Sn , ^{131}I , ^{166}Ho , ^{177}Lu , ^{186}Re , ^{188}Re , ^{194}Ir , ^{199}Au (Illidge, T.M. & Brock, S. 2000, *Curr Pharm. Design* 6: 1399). In humans, clinical trials are underway utilizing a yttrium-90 conjugated anti-CD20 antibody for B cell lymphomas (*Cancer Chemother Pharmacol* 48(Suppl 1):S91-S95 (2001)).

25 General techniques have been previously described (U.S. Patent Nos. 6,306,393 and 5,057,313, Shih, *et al.*, *Int. J. Cancer* 41:832-839 (1988); Shih, *et al.*, *Int. J. Cancer* 46:1101-1106 (1990)). The general method involves reacting an antibody component having an oxidized carbohydrate portion with a carrier polymer that has at least one free amine function and that is loaded with a plurality of drug, toxin, chelator, boron addends, 30 or other therapeutic agent. This reaction results in an initial Schiff base (imine) linkage, which can be stabilized by reduction to a secondary amine to form the final conjugate.

The carrier polymer is preferably an aminodextran or polypeptide of at least 50 amino acid residues, although other substantially equivalent polymer carriers can also be used. Preferably, the final immunoconjugate is soluble in an aqueous solution, such as mammalian serum, for ease of administration and effective targeting for use in therapy.

5 Thus, solubilizing functions on the carrier polymer will enhance the serum solubility of the final immunoconjugate. In particular, an aminodextran will be preferred.

The process for preparing an immunoconjugate with an aminodextran carrier typically begins with a dextran polymer, advantageously a dextran of average molecular weight of about 10,000-100,000. The dextran is reacted with an oxidizing agent to effect 10 a controlled oxidation of a portion of its carbohydrate rings to generate aldehyde groups. The oxidation is conveniently effected with glycolytic chemical reagents such as NaIO₄, according to conventional procedures. The oxidized dextran is then reacted with a polyamine, preferably a diamine, and more preferably, a mono- or polyhydroxy diamine. Suitable amines include ethylene diamine, propylene diamine, or other like 15 polymethylene diamines, diethylene triamine or like polyamines, 1,3-diamino-2-hydroxypropane, or other like hydroxylated diamines or polyamines, and the like. An excess of the amine relative to the aldehyde groups of the dextran is used to ensure substantially complete conversion of the aldehyde functions to Schiff base groups. A reducing agent, such as NaBH₄, NaBH₃ CN or the like, is used to effect reductive 20 stabilization of the resultant Schiff base intermediate. The resultant adduct can be purified by passage through a conventional sizing column or ultrafiltration membrane to remove cross-linked dextrans. Other conventional methods of derivatizing a dextran to introduce amine functions can also be used, e.g., reaction with cyanogen bromide, followed by reaction with a diamine.

25 The aminodextran is then reacted with a derivative of the particular drug, toxin, chelator, immunomodulator, boron addend, or other therapeutic agent to be loaded, in an activated form, preferably, a carboxyl-activated derivative, prepared by conventional means, e.g., using dicyclohexylcarbodiimide (DCC) or a water soluble variant thereof, to form an intermediate adduct. Alternatively, polypeptide toxins such as pokeweed 30 antiviral protein or ricin A-chain, and the like, can be coupled to aminodextran by

glutaraldehyde condensation or by reaction of activated carboxyl groups on the protein with amines on the aminodextran.

Chelators for radiometals or magnetic resonance enhancers are well-known in the art. Typical are derivatives of ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA). These chelators typically have groups on the side chain by which the chelator can be attached to a carrier. Such groups include, e.g., benzylisothiocyanate, by which the DTPA or EDTA can be coupled to the amine group of a carrier. Alternatively, carboxyl groups or amine groups on a chelator can be coupled to a carrier by activation or prior derivatization and then coupling, all by well-known means.

Boron addends, such as carboranes, can be attached to antibody components by conventional methods. For example, carboranes can be prepared with carboxyl functions on pendant side chains, as is well known in the art. Attachment of such carboranes to a carrier, e.g., aminodextran, can be achieved by activation of the carboxyl groups of the carboranes and condensation with amines on the carrier to produce an intermediate conjugate. Such intermediate conjugates are then attached to antibody components to produce therapeutically useful immunoconjugates, as described below.

A polypeptide carrier can be used instead of aminodextran, but the polypeptide carrier should have at least 50 amino acid residues in the chain, preferably 100-5000 amino acid residues. At least some of the amino acids should be lysine residues or glutamate or aspartate residues. The pendant amines of lysine residues and pendant carboxylates of glutamine and aspartate are convenient for attaching a drug, toxin, immunomodulator, chelator, boron addend or other therapeutic agent. Examples of suitable polypeptide carriers include polylysine, polyglutamic acid, polyaspartic acid, copolymers thereof, and mixed polymers of these amino acids and others, e.g., serines, to confer desirable solubility properties on the resultant loaded carrier and immunoconjugate.

Conjugation of the intermediate conjugate with the antibody component is effected by oxidizing the carbohydrate portion of the antibody component and reacting the resulting aldehyde (and ketone) carbonyls with amine groups remaining on the carrier after loading with a drug, toxin, chelator, immunomodulator, boron addend, or other

therapeutic agent. Alternatively, an intermediate conjugate can be attached to an oxidized antibody component via amine groups that have been introduced in the intermediate conjugate after loading with the therapeutic agent. Oxidation is conveniently effected either chemically, e.g., with NaIO_4 or other glycolytic reagent, or

5 enzymatically, e.g., with neuraminidase and galactose oxidase. In the case of an aminodextran carrier, not all of the amines of the aminodextran are typically used for loading a therapeutic agent. The remaining amines of aminodextran condense with the oxidized antibody component to form Schiff base adducts, which are then reductively stabilized, normally with a borohydride reducing agent.

10 Analogous procedures are used to produce other immunoconjugates according to the invention. Loaded polypeptide carriers preferably have free lysine residues remaining for condensation with the oxidized carbohydrate portion of an antibody component. Carboxyls on the polypeptide carrier can, if necessary, be converted to amines by, e.g., activation with DCC and reaction with an excess of a diamine.

15 The final immunoconjugate is purified using conventional techniques, such as sizing chromatography on Sephadex S-300 or affinity chromatography using one or more SEQ ID NO: 2 or 4 epitopes.

Alternatively, immunoconjugates can be prepared by directly conjugating an antibody component with a therapeutic agent. The general procedure is analogous to the

20 indirect method of conjugation except that a therapeutic agent is directly attached to an oxidized antibody component. It will be appreciated that other therapeutic agents can be substituted for the chelators described herein. Those of skill in the art will be able to devise conjugation schemes without undue experimentation.

As a further illustration, a therapeutic agent can be attached at the hinge region of

25 a reduced antibody component via disulfide bond formation. For example, the tetanus toxoid peptides can be constructed with a single cysteine residue that is used to attach the peptide to an antibody component. As an alternative, such peptides can be attached to the antibody component using a heterobifunctional cross-linker, such as N-succinyl 3-(2-pyridylthio)propionate (SPDP) (Yu, et al., *Int. J. Cancer* 56:244 (1994)). General

30 techniques for such conjugation have been previously described (Wong, *Chemistry of Protein Conjugation and Cross-linking*, CRC Press (1991); Upeslakis, et al., pp. 187-230

in, *Monoclonal Antibodies Principles and Applications*, Eds. Birch *et al.*, Wiley-Liss, Inc. (1995); Price, pp. 60-84 in, *Monoclonal Antibodies: Production, Engineering and Clinical Applications* Eds. Ritter, *et al.*, Cambridge University Press (1995)).

As described above, carbohydrate moieties in the Fc region of an antibody can be used to conjugate a therapeutic agent. However, the Fc region may be absent if an antibody fragment is used as the antibody component of the immunoconjugate. Nevertheless, it is possible to introduce a carbohydrate moiety into the light chain variable region of an antibody or antibody fragment (Leung, *et al.*, *J. Immunol.* 154:5919-5926 (1995); U.S. Pat. No. 5,443,953). The engineered carbohydrate moiety is then used to attach a therapeutic agent.

In addition, those of skill in the art will recognize numerous possible variations of the conjugation methods. For example, the carbohydrate moiety can be used to attach polyethyleneglycol in order to extend the half-life of an intact antibody, or antigen-binding fragment thereof, in blood, lymph, or other extracellular fluids. Moreover, it is possible to construct a "divalent immunoconjugate" by attaching therapeutic agents to a carbohydrate moiety and to a free sulphydryl group. Such a free sulphydryl group may be located in the hinge region of the antibody component.

(3) ANTI-SEQ ID NO: 2 OR 4 ANTIBODY FUSION PROTEINS

When the therapeutic agent to be conjugated to the antibody is a protein, the present invention contemplates the use of fusion proteins comprising one or more anti-SEQ ID NO: 2 or 4 antibody moieties and an immunomodulator or toxin moiety. Methods of making antibody fusion proteins have been previously described (U.S. Patent No. 6,306,393). Antibody fusion proteins comprising an interleukin-2 moiety have also been previously disclosed (Boleti, *et al.*, *Ann. Oncol.* 6:945 (1995), Nicolet, *et al.*, *Cancer Gene Ther.* 2:161 (1995), Becker, *et al.*, *Proc. Nat'l Acad. Sci. USA* 93:7826 (1996), Hank, *et al.*, *Clin. Cancer Res.* 2:1951 (1996), Hu, *et al.*, *Cancer Res.* 56:4998 (1996)). In addition, Yang, *et al.*, *Hum. Antibodies Hybridomas* 6:129 (1995), describe a fusion protein that includes an F(ab')₂ fragment and a tumor necrosis factor alpha moiety.

Methods of making antibody-toxin fusion proteins in which a recombinant molecule comprises one or more antibody components and a toxin or chemotherapeutic

agent also are known to those of skill in the art. For example, antibody-*Pseudomonas* exotoxin A fusion proteins have been described (Chaudhary, *et al.*, *Nature* 339:394 (1989), Brinkmann, *et al.*, *Proc. Nat'l Acad. Sci. USA* 88:8616 (1991), Batra, *et al.*, *Proc. Natl. Acad. Sci. USA* 89:5867 (1992), Friedman, *et al.*, *J. Immunol.* 150:3054 (1993), 5 Wels, *et al.*, *Int. J. Can.* 60:137 (1995), Fominaya *et al.*, *J. Biol. Chem.* 271:10560 (1996), Kuan, *et al.*, *Biochemistry* 35:2872 (1996), Schmidt, *et al.*, *Int. J. Can.* 65:538 (1996)). Antibody-toxin fusion proteins containing a diphtheria toxin moiety have been described (Kreitman, *et al.*, *Leukemia* 7:553 (1993), Nicholls, *et al.*, *J. Biol. Chem.* 268:5302 (1993), Thompson, *et al.*, *J. Biol. Chem.* 270:28037 (1995), and Vallera, *et al.*, 10 *Blood* 88:2342 (1996). Deonarain *et al.* (*Tumor Targeting* 1:177 (1995)), have described an antibody-toxin fusion protein having an RNase moiety, while Linardou, *et al.* (*Cell Biophys.* 24-25:243 (1994)), produced an antibody-toxin fusion protein comprising a DNase I component. Gelonin and *Staphylococcal* enterotoxin-A have been used as the toxin moieties in antibody-toxin fusion proteins (Wang, *et al.*, Abstracts of the 209th 15 ACS National Meeting, Anaheim, Calif., Apr. 2-6, 1995, Part 1, BIOT005; Dohlsten, *et al.*, *Proc. Nat'l Acad. Sci. USA* 91:8945 (1994)).

DISEASES AMENABLE TO ANTI-SEQ ID NO: 2 OR 4 IMMUNOTARGETING

In one aspect, the present invention provides reagents and methods useful for 20 treating diseases and conditions wherein cells associated with the disease or disorder express SEQ ID NO: 2 or 4. These diseases can include cancers, and other hyperproliferative conditions, such as hyperplasia, psoriasis, contact dermatitis, immunological disorders, and infertility. Whether the cells associated with a disease or condition express SEQ ID NO: 2 or 4 can be determined using the diagnostic methods 25 described herein.

Comparisons of SEQ ID NO: 2 or 4-encoding mRNA and protein expression levels between diseased cells, tissue or fluid (blood, lymphatic fluid, etc.) and corresponding normal samples are made to determine if the patient will be responsive to SEQ ID NO: 2 or 4 immunotherapy. Methods for detecting and quantifying the 30 expression of SEQ ID NO: 2 or 4-encoding mRNA or protein use standard nucleic acid and protein detection and quantitation techniques that are well known in the art and are

described in Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, NY (1989) or Ausubel, *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY (1989), both of which are incorporated herein by reference in their entirety. Standard methods for the detection and

5 quantification of CD94/Hy1 mRNA include *in situ* hybridization using labeled SEQ ID NO: 2 or 4 riboprobes (Gemou-Engesaeth, *et al.*, *Pediatrics* 109: E24-E32 (2002)), Northern blot and related techniques using SEQ ID NO: 2 or 4 polynucleotide probes (Kunzli, *et al.*, *Cancer* 94: 228 (2002)), RT-PCR analysis using SEQ ID NO: 2 or 4-specific primers (Angchaikisiri, *et al.*, *Blood* 99:130 (2002)), and other amplification

10 detection methods, such as branched chain DNA solution hybridization assay (Jardi, *et al.*, *J. Viral Hepat.* 8:465-471 (2001)), transcription-mediated amplification (Kimura, *et al.*, *J. Clin. Microbiol.* 40:439-445 (2002)), microarray products, such as oligos, cDNAs, and monoclonal antibodies, and real-time PCR (Simpson, *et al.*, *Molec. Vision*, 6:178-183 (2000)). Standard methods for the detection and quantification of SEQ ID NO: 2 or 4

15 protein include western blot analysis (Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, NY (1989), Ausubel, *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY (1989)), immunocytochemistry (Racila, *et al.*, *Proc. Natl. Acad. Sci. USA* 95:4589-4594 (1998)), and a variety of immunoassays, including enzyme-linked immunosorbant assay (ELISA),

20 radioimmuno assay (RIA), and specific enzyme immunoassay (EIA) (Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, NY (1989), Ausubel, *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY (1989)). Peripheral blood cells can also be analyzed for SEQ ID NO: 2 or 4 expression using flow cytometry using, for example, immunomagnetic beads specific for

25 SEQ ID NO: 2 or 4 (Racila, *et al.*, *Proc. Natl. Acad. Sci. USA* 95:4589-4594 (1998)) or biotinylated SEQ ID NO: 2 or 4 antibodies (Soltys, *et al.*, *J. Immunol.* 168:1903 (2002)). Tumor aggressiveness can be gauged by determining the levels of SEQ ID NO: 2 or 4 protein or mRNA in tumor cells compared to the corresponding normal cells (Orlandi, *et al.*, *Cancer Res.* 62:567 (2002)). In one embodiment, the disease or disorder is a cancer.

30 Cancer, a leading cause of death in the United States, causes over a half-million deaths annually. As the population ages, the numbers of deaths due to cancer are expected to

rise significantly. Cancer is a general term and encompasses various types of malignant neoplasms, most of which invade surrounding tissues, may metastasize to several sites, and are likely to recur after attempted removal and to cause death of the patient unless adequately treated. Cancer can develop in any tissue of any organ at any age. Once a 5 cancer diagnosis is made, treatment decisions are paramount. Successful therapy focuses on the primary tumor and its metastases. Various types of cancer treatments have been developed to improve the survival and quality of life of cancer patients. Advances in cancer treatment include new cytotoxic agents and new surgical and radiotherapy techniques. However, many of these treatments have substantial emotional and physical 10 drawbacks. Furthermore, treatment failure remains a common occurrence. Such shortcomings have driven cancer researchers and caregivers to develop new and effective ways of treating cancer.

The cancers treatable by methods of the present invention preferably occur in mammals. Mammals include, for example, humans and other primates, as well as pet or 15 companion animals such as dogs and cats, laboratory animals such as rats, mice and rabbits, and farm animals such as horses, pigs, sheep, and cattle.

Tumors or neoplasms include growths of tissue cells in which the multiplication of the cells is uncontrolled and progressive. Some such growths are benign, but others are termed "malignant" and may lead to death of the organism. Malignant neoplasms or 20 "cancers" are distinguished from benign growths in that, in addition to exhibiting aggressive cellular proliferation, they may invade surrounding tissues and metastasize. Moreover, malignant neoplasms are characterized in that they show a greater loss of differentiation (greater "dedifferentiation"), and greater loss of their organization relative to one another and their surrounding tissues. This property is also called "anaplasia."

25 Neoplasms treatable by the present invention also include solid phase tumors/malignancies, *i.e.*, carcinomas, locally advanced tumors and human soft tissue sarcomas. Carcinomas include those malignant neoplasms derived from epithelial cells that infiltrate (invade) the surrounding tissues and give rise to metastatic cancers, including lymphatic metastases. Adenocarcinomas are carcinomas derived from 30 glandular tissue, or which form recognizable glandular structures. Another broad category of cancers includes sarcomas, which are tumors whose cells are embedded in a

fibrillar or homogeneous substance like embryonic connective tissue. The invention also enables treatment of cancers of the myeloid or lymphoid systems, including leukemias, lymphomas and other cancers that typically do not present as a tumor mass, but are distributed in the vascular or lymphoreticular systems.

5 The type of cancer or tumor cells that may be amenable to treatment according to the invention include, for example, acute lymphocytic leukemia, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, chronic myelocytic leukemia, cutaneous T-cell lymphoma, hairy cell leukemia, acute myeloid leukemia, erythroleukemia, chronic myeloid (granulocytic) leukemia, Hodgkin's disease, and non-Hodgkin's lymphoma,

10 gastrointestinal cancers including esophageal cancer, stomach cancer, colon cancer, colorectal cancer, polyps associated with colorectal neoplasms, pancreatic cancer and gallbladder cancer, cancer of the adrenal cortex, ACTH-producing tumor, bladder cancer, brain cancer including intrinsic brain tumors, neuroblastomas, astrocytic brain tumors, gliomas, and metastatic tumor cell invasion of the central nervous system,

15 Ewing's sarcoma, head and neck cancer including mouth cancer and larynx cancer, kidney cancer including renal cell carcinoma, liver cancer, lung cancer including small and non-small cell lung cancers, malignant peritoneal effusion, malignant pleural effusion, skin cancers including malignant melanoma, tumor progression of human skin keratinocytes, squamous cell carcinoma, basal cell carcinoma, and hemangiopericytoma,

20 mesothelioma, Kaposi's sarcoma, bone cancer including osteomas and sarcomas such as fibrosarcoma and osteosarcoma, cancers of the female reproductive tract including uterine cancer, endometrial cancer, ovarian cancer, ovarian (germ cell) cancer and solid tumors in the ovarian follicle, vaginal cancer, cancer of the vulva, and cervical cancer; breast cancer (small cell and ductal), penile cancer, prostate cancer, retinoblastoma,

25 testicular cancer, thyroid cancer, trophoblastic neoplasms, and Wilms' tumor.

The invention is particularly illustrated herein in reference to treatment of certain types of experimentally defined cancers. In these illustrative treatments, standard state-of-the-art *in vitro* and *in vivo* models have been used. These methods can be used to identify agents that can be expected to be efficacious in *in vivo* treatment regimens.

30 However, it will be understood that the method of the invention is not limited to the treatment of these tumor types, but extends to any cancer derived from any organ system.

As demonstrated in the Examples, SEQ ID NO: 2 or 4 is highly expressed in primary B cells and B-cell related disorders. Leukemias can result from uncontrolled B cell proliferation initially within the bone marrow before disseminating to the peripheral blood, spleen, lymph nodes and finally to other tissues. Uncontrolled B cell proliferation 5 also may result in the development of lymphomas that arise within the lymph nodes and then spread to the blood and bone marrow. Immunotargeting SEQ ID NO: 2 or 4 is used in treating B and T cell malignancies, leukemias, lymphomas and myelomas including but not limited to multiple myeloma, Burkitt's lymphoma, cutaneous B cell lymphoma, primary follicular cutaneous B cell lymphoma, B lineage acute lymphoblastic leukemia 10 (ALL), B cell non-Hodgkin's lymphoma (NHL), B cell chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia, hairy cell leukemia (HCL), splenic marginal zone lymphoma, diffuse large B cell lymphoma, prolymphocytic leukemia (PLL), lymphoplasma cytoid lymphoma, mantle cell lymphoma, mucosa-associated lymphoid tissue (MALT) lymphoma, primary thyroid lymphoma, intravascular malignant 15 lymphomatosis, splenic lymphoma, Hodgkin's Disease, and intragraft angiotropic large-cell lymphoma. Expression of SEQ ID NO: 2 or 4 has also been demonstrated in Examples 1 and 2 in myeloid leukemia cell lines and tissue, T cell leukemia cell lines and T cell lymphoma tissues and may be treated with anti-SEQ ID NO: 2 or 4 antibodies. Other diseases that may be treated by the methods of the present invention include 20 multicentric Castleman's disease, primary amyloidosis, Franklin's disease, Seligmann's disease, primary effusion lymphoma, post-transplant lymphoproliferative disease (PTLD) [associated with EBV infection], paraneoplastic pemphigus, chronic lymphoproliferative disorders, X-linked lymphoproliferative syndrome (XLP), acquired angioedema, angioimmunoblastic lymphadenopathy with dysproteinemia, Herman's syndrome, post- 25 splenectomy syndrome, congenital dyserythropoietic anemia type III, lymphoma-associated hemophagocytic syndrome (LAHS), necrotizing ulcerative stomatitis, Kikuchi's disease, lymphomatoid granulomatosis, Richter's syndrome, polycythemic vera (PV), Gaucher's disease, Gougerot-Sjögren syndrome, Kaposi's sarcoma, cerebral lymphoplasmacytic proliferation (Bind and Neel syndrome), X-linked 30 lymphoproliferative disorders, pathogen associated disorders such as mononucleosis

(Epstein Barr Virus), lymphoplasma cellular disorders, post-transplantational plasma cell dyscrasias, and Good's syndrome.

Autoimmune diseases can be associated with hyperactive B and T cell activity that results in autoantibody production and cell-mediated immunity. Inhibition of the development of autoantibody-producing cells or proliferation of such cells may be therapeutically effective in decreasing the levels of autoantibodies in autoimmune diseases including but not limited to systemic lupus erythematosus, Crohn's Disease, graft-verses-host disease, Graves' disease, myasthenia gravis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, asthma, cryoglobulinemia, primary biliary sclerosis, pernicious anemia, Waldenstrom macroglobulinemia, hyperviscosity syndrome, macroglobulinemia, cold agglutinin disease, monoclonal gammopathy of undetermined origin, anetoderma and POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M component, skin changes), connective tissue disease, multiple sclerosis, cystic fibrosis, rheumatoid arthritis, autoimmune pulmonary inflammation, psoriasis, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, autoimmune inflammatory eye disease, Goodpasture's disease, Rasmussen's encephalitis, dermatitis herpetiformis, thyoma, autoimmune polyglandular syndrome type 1, primary and secondary membranous nephropathy, cancer-associated retinopathy, autoimmune hepatitis type 1, mixed cryoglobulinemia with renal involvement, cystoid macular edema, endometriosis, IgM polyneuropathy (including Hyper IgM syndrome), demyelinating diseases, angiogenesis, and monoclonal gammopathy.

Immunotargeting SEQ ID NO: 2 or 4 may also be useful in the treatment of allergic reactions and conditions e.g., anaphylaxis, serum sickness, drug reactions, food allergies, insect venom allergies, mastocytosis, allergic rhinitis, hypersensitivity pneumonitis, urticaria, angioedema, eczema, atopic dermatitis, allergic contact dermatitis, erythema multiforme, Stevens-Johnson syndrome, allergic conjunctivitis, atopic keratoconjunctivitis, venereal keratoconjunctivitis, giant papillary conjunctivitis, allergic gastroenteropathy, inflammatory bowel disorder (IBD), and contact allergies, such as asthma (particularly allergic asthma), or other respiratory problems.

30

ADMINISTRATION

The anti-SEQ ID NO: 2 or 4 monoclonal antibodies used in the practice of a method of the invention may be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material which when combined with the anti-SEQ ID NO: 2 or 4 antibodies retains the anti-tumor 5 function of the antibody and is nonreactive with the subject's immune systems. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like.

The anti-SEQ ID NO: 2 or 4 antibody formulations may be administered via any route capable of delivering the antibodies to the tumor site. Potentially effective routes of 10 administration include, but are not limited to, intravenous, intraperitoneal, intramuscular, intratumor, intradermal, and the like. The preferred route of administration is by intravenous injection. A preferred formulation for intravenous injection comprises anti-SEQ ID NO: 2 or 4 mAbs in a solution of preserved bacteriostatic water, sterile unpreserved water, and/or diluted in polyvinylchloride or polyethylene bags containing 15 0.9% sterile sodium chloride for Injection, USP. The anti-SEQ ID NO: 2 or 4 mAb preparation may be lyophilized and stored as a sterile powder, preferably under vacuum, and then reconstituted in bacteriostatic water containing, for example, benzyl alcohol preservative, or in sterile water prior to injection.

Treatment will generally involve the repeated administration of the anti-SEQ ID 20 NO: 2 or 4 antibody preparation via an acceptable route of administration such as intravenous injection (IV), typically at a dose in the range of about 0.1 to about 10 mg/kg body weight; however other exemplary doses in the range of 0.01 mg/kg to about 100 mg/kg are also contemplated. Doses in the range of 10-500 mg mAb per week may be effective and well tolerated. Rituximab (Rituxan®), a chimeric CD20 antibody used to 25 treat B-cell lymphoma, non-Hodgkin's lymphoma, and relapsed indolent lymphoma, is typically administered at 375 mg/m² by IV infusion once a week for 4 to 8 doses. Sometimes a second course is necessary, but no more than 2 courses are allowed. An effective dosage range for Rituxan® would be 50 to 500 mg/m² (Maloney, *et al.*, *Blood* 84: 2457-2466 (1994); Davis, *et al.*, *J. Clin. Oncol.* 18: 3135-3143 (2000)). Based on 30 clinical experience with Trastuzumab (Herceptin®), a humanized monoclonal antibody used to treat HER2(human epidermal growth factor 2)-positive metastatic breast cancer

(Slamon, *et al.*, *Mol Cell Biol.* 9: 1165 (1989)), an initial loading dose of approximately 4 mg/kg patient body weight IV followed by weekly doses of about 2 mg/kg IV of the anti-SEQ ID NO: 2 or 4 mAb preparation may represent an acceptable dosing regimen (Slamon, *et al.*, *N. Engl. J. Med.* 344: 783(2001)). Preferably, the initial loading dose is 5 administered as a 90 minute or longer infusion. The periodic maintenance dose may be administered as a 30 minute or longer infusion, provided the initial dose was well tolerated. However, as one of skill in the art will understand, various factors will influence the ideal dose regimen in a particular case. Such factors may include, for example, the binding affinity and half life of the mAb or mAbs used, the degree of SEQ 10 ID NO: 2 or 4 overexpression in the patient, the extent of circulating shed SEQ ID NO: 2 or 4 antigen, the desired steady-state antibody concentration level, frequency of treatment, and the influence of chemotherapeutic agents used in combination with the treatment method of the invention.

Treatment can also involve anti-SEQ ID NO: 2 or 4 antibodies conjugated to 15 radioisotopes. Studies using radiolabeled-anticarcinoembryonic antigen (anti-CEA) monoclonal antibodies, provide a dosage guideline for tumor regression of 2-3 infusions of 30-80 mCi/m² (Behr, *et al.* *Clin. Cancer Res.* 5(10 Suppl.): 3232s-3242s (1999), Juweid, *et al.*, *J. Nucl. Med.* 39:34-42 (1998)).

Alternatively, dendritic cells transfected with mRNA encoding SEQ ID NO: 2 or 20 4 can be used as a vaccine to stimulate T-cell mediated anti-tumor responses. Studies with dendritic cells transfected with prostate-specific antigen mRNA suggest a 3 cycles of intravenous administration of 1×10^7 – 5×10^7 cells for 2-6 weeks concomitant with an intradermal injection of 10^7 cells may provide a suitable dosage regimen (Heiser, *et al.*, *J. Clin. Invest.* 109:409-417 (2002); Hadzantonis and O'Neill, *Cancer Biother. Radiopharm.* 1:11-22 (1999)). Other exemplary doses of between 1×10^5 to 1×10^9 or 25 1×10^6 to 1×10^8 cells are also contemplated.

Naked DNA vaccines using plasmids encoding SEQ ID NO: 2 or 4 can induce an 30 immunologic anti-tumor response. Administration of naked DNA by direct injection into the skin and muscle is not associated with limitations encountered using viral vectors, such as the development of adverse immune reactions and risk of insertional mutagenesis (Hengge, *et al.*, *J. Invest. Dermatol.* 116:979 (2001)). Studies have shown

that direct injection of exogenous cDNA into muscle tissue results in a strong immune response and protective immunity (Ilan, *Curr. Opin. Mol. Ther.* 1:116-120 (1999)). Physical (gene gun, electroporation) and chemical (cationic lipid or polymer) approaches have been developed to enhance efficiency and target cell specificity of gene transfer by plasmid DNA (Nishikawa and Huang, *Hum. Gene Ther.* 12:861-870 (2001)). Plasmid DNA can also be administered to the lungs by aerosol delivery (Densmore, *et al.*, *Mol. Ther.* 1:180-188 (2000)). Gene therapy by direct injection of naked or lipid - coated plasmid DNA is envisioned for the prevention, treatment, and cure of diseases such as cancer, acquired immunodeficiency syndrome, cystic fibrosis, cerebrovascular disease, and hypertension (Prazeres, *et al.*, *Trends Biotechnol.* 17:169-174 (1999); Weihl, *et al.*, *Neurosurgery* 44:239-252 (1999)). HIV-1 DNA vaccine dose-escalating studies indicate administration of 30-300 µg/dose as a suitable therapy (Weber, *et al.*, *Eur. J. Clin. Microbiol. Infect. Dis.* 20: 800). Naked DNA injected intracerebrally into the mouse brain was shown to provide expression of a reporter protein, wherein expression was dose-dependent and maximal for 150 µg DNA injected (Schwartz, *et al.*, *Gene Ther.* 3:405-411 (1996)). Gene expression in mice after intramuscular injection of nanospheres containing 1 microgram of beta-galactosidase plasmid was greater and more prolonged than was observed after an injection with an equal amount of naked DNA or DNA complexed with Lipofectamine (Truong, *et al.*, *Hum. Gene Ther.* 9:1709-1717 (1998)).

In a study of plasmid-mediated gene transfer into skeletal muscle as a means of providing a therapeutic source of insulin, wherein four plasmid constructs comprising a mouse furin cDNA transgene and rat proinsulin cDNA were injected into the calf muscles of male Balb/c mice, the optimal dose for most constructs was 100 micrograms plasmid DNA (Kon, *et al.* *J. Gene Med.* 1:186-194 (1999)). Other exemplary doses of 1-1000 µg/dose or 10-500 µg/dose are also contemplated.

Optimally, patients should be evaluated for the level of circulating shed SEQ ID NO: 2 or 4 antigen in serum in order to assist in the determination of the most effective dosing regimen and related factors. Such evaluations may also be used for monitoring purposes throughout therapy, and may be useful to gauge therapeutic success in combination with evaluating other parameters.

(1) SEQ ID NO: 2 OR 4 TARGETING COMPOSITIONS

Compositions for targeting SEQ ID NO: 2 or 4-expressing cells are within the scope of the present invention. Pharmaceutical compositions comprising antibodies are described in detail in, for example, US Patent No. 6,171,586, to Lam *et al.*, issued 5 January 9, 2001. Such compositions comprise a therapeutically or prophylactically effective amount an antibody, or a fragment, variant, derivative or fusion thereof as described herein, in admixture with a pharmaceutically acceptable agent. Typically, the SEQ ID NO: 2 or 4 immunotargeting agent will be sufficiently purified for administration to an animal.

10 The pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine);

15 antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, other organic acids); bulking agents (such as mannitol or glycine), chelating agents [such as ethylenediamine tetraacetic acid (EDTA)]; complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers;

20 monosaccharides; disaccharides and other carbohydrates (such as glucose, mannose, or dextrans); proteins (such as serum albumin, gelatin or immunoglobulins); coloring; flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic

25 acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability

30 enhancing agents (sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides (preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles;

diluents; excipients and/or pharmaceutical adjuvants. (*Remington's Pharmaceutical Sciences*, 18th Edition, Ed. A.R. Gennaro, Mack Publishing Company, (1990)).

The optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage. See, for example, *Remington's Pharmaceutical Sciences, supra*. Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the SEQ ID NO: 2 or 4 immunotargeting agent.

The primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefor. In one embodiment of the present invention, SEQ ID NO: 2 or 4 immunotargeting agent compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (*Remington's Pharmaceutical Sciences, supra*) in the form of a lyophilized cake or an aqueous solution. Further, the binding agent product may be formulated as a lyophilizate using appropriate excipients such as sucrose.

The pharmaceutical compositions can be selected for parenteral delivery. Alternatively, the compositions may be selected for inhalation or for delivery through the digestive tract, such as orally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art. The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at slightly lower pH, typically within a pH range of from about 5 to about 8. When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the SEQ ID NO: 2 or 4 immunotargeting agent in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which a SEQ ID NO: 2

or 4 immunotargeting agent is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered via a depot injection. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

In another aspect, pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

In another embodiment, a pharmaceutical composition may be formulated for inhalation. For example, a SEQ ID NO: 2 or 4 immunotargeting agent may be formulated as a dry powder for inhalation. Polypeptide or nucleic acid molecule inhalation solutions may also be formulated with a propellant for aerosol delivery. In yet another embodiment, solutions may be nebulized. Pulmonary administration is further described in PCT Application No. PCT/US94/001875, which describes pulmonary delivery of chemically modified proteins.

It is also contemplated that certain formulations may be administered orally. In one embodiment of the present invention, SEQ ID NO: 2 or 4 immunotargeting agents that are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of the formulation at

the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the binding agent molecule. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be
5 employed.

Pharmaceutical compositions for oral administration can also be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like,
10 for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as
15 sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethylcellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a
20 salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may
25 be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations that can be used orally also include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or
30 binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the SEQ ID NO: 2 or 4 immunotargeting agent

may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Another pharmaceutical composition may involve an effective quantity of SEQ ID NO: 2 or 4 immunotargeting agent in a mixture with non-toxic excipients that are 5 suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or other appropriate vehicle, solutions can be prepared in unit dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

10 Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations involving SEQ ID NO: 2 or 4 immunotargeting agents in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the 15 art. See, for example, PCT/US93/00829 that describes controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions. Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (U.S. Patent No. 3,773,919, EP 58,481), 20 copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, *Biopolymers*, 22:547-556 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, *J Biomed Mater Res*, 15:167-277, (1981)) and (Langer *et al.*, *Chem Tech*, 12:98-105(1982)), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomes, which can be 25 prepared by any of several methods known in the art. See e.g., Epstein, *et al.*, *Proc Natl Acad Sci (USA)*, 82:3688-3692 (1985); EP 36,676; EP 88,046; EP 143,949.

The pharmaceutical composition to be used for *in vivo* administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method may 30 be conducted either prior to or following lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in

solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Once the pharmaceutical composition has been formulated, it may be stored in 5 sterile vials as a solution, suspension, gel, emulsion, solid, or a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

In a specific embodiment, the present invention is directed to kits for producing a single-dose administration unit. The kits may each contain both a first container having a 10 dried SEQ ID NO: 2 or 4 immunotargeting agent and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes).

(2) DOSAGE

An effective amount of a pharmaceutical composition to be employed 15 therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which SEQ 20 ID NO: 2 or 4 immunotargeting agent is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 mg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage may range from 0.1 mg/kg up to about 100 25 mg/kg; or 0.01 mg/kg to 1 g/kg; or 1 mg/kg up to about 100 mg/kg or 5 mg/kg up to about 100 mg/kg. In other embodiments, the dosage may range from 10 mCi to 100 mCi per dose for radioimmunotherapy, from about 1×10^7 – 5×10^7 cells or 1×10^5 to 1×10^9 cells or 1×10^6 to 1×10^8 cells per injection or infusion, or from 30 μ g to 300 μ g naked DNA per dose or 1-1000 μ g/dose or 10-500 μ g/dose, depending on the factors listed above. 30 For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models such as mice, rats, rabbits, dogs, or pigs.

An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The exact dosage will be determined in light of factors related to the subject

5 requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active compound or to maintain the desired effect. Factors that may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting

10 pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

The frequency of dosing will depend upon the pharmacokinetic parameters of the SEQ ID NO: 2 or 4 immunotargeting agent in the formulation used. Typically, a composition is administered until a dosage is reached that achieves the desired effect.

15 The composition may therefore be administered as a single dose, or as multiple doses (at the same or different concentrations/dosages) over time, or as a continuous infusion. Further refinement of the appropriate dosage is routinely made. Appropriate dosages may be ascertained through use of appropriate dose-response data.

20 (3) ROUTES OF ADMINISTRATION

The route of administration of the pharmaceutical composition is in accord with known methods, e.g. orally, through injection by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intra-ocular, intra-arterial, intraportal, intralesional routes, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, urethral, vaginal, or rectal means, by sustained release systems or by implantation devices. Where desired, the compositions may be administered by bolus injection or continuously by infusion, or by implantation device.

Alternatively or additionally, the composition may be administered locally via

30 implantation of a membrane, sponge, or another appropriate material on to which the SEQ ID NO: 2 or 4 immunotargeting agent has been absorbed or encapsulated. Where an

implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of the SEQ ID NO: 2 or 4 immunotargeting agent may be via diffusion, timed-release bolus, or continuous administration.

In some cases, it may be desirable to use pharmaceutical compositions in an *ex vivo* manner. In such instances, cells, tissues, or organs that have been removed from the patient are exposed to the pharmaceutical compositions after which the cells, tissues and/or organs are subsequently implanted back into the patient.

In other cases, a SEQ ID NO: 2 or 4 immunotargeting agent can be delivered by implanting certain cells that have been genetically engineered to express and secrete the polypeptide. Such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. Optionally, the cells may be immortalized. In order to decrease the chance of an immunological response, the cells may be encapsulated to avoid infiltration of surrounding tissues. The encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

COMBINATION THERAPY

SEQ ID NO: 2 or 4 targeting agents of the invention can be utilized in combination with other therapeutic agents. These other therapeutics include, for example radiation treatment, chemotherapeutic agents, as well as other growth factors.

In one embodiment, anti-SEQ ID NO: 2 or 4 antibody is used as a radiosensitizer. In such embodiments, the anti-SEQ ID NO: 2 or 4 antibody is conjugated to a radiosensitizing agent. The term "radiosensitizer," as used herein, is defined as a molecule, preferably a low molecular weight molecule, administered to animals in therapeutically effective amounts to increase the sensitivity of the cells to be radiosensitized to electromagnetic radiation and/or to promote the treatment of diseases that are treatable with electromagnetic radiation. Diseases that are treatable with electromagnetic radiation include neoplastic diseases, benign and malignant tumors, and cancerous cells.

The terms "electromagnetic radiation" and "radiation" as used herein include, but are not limited to, radiation having the wavelength of 10^{-20} to 100 meters. Preferred embodiments of the present invention employ the electromagnetic radiation of: gamma-radiation (10^{-20} to 10^{-13} m), X-ray radiation (10^{-12} to 10^{-9} m), ultraviolet light (10 nm to 400 nm), visible light (400 nm to 700 nm), infrared radiation (700 nm to 1.0 mm), and microwave radiation (1 mm to 30 cm).

Radiosensitizers are known to increase the sensitivity of cancerous cells to the toxic effects of electromagnetic radiation. Many cancer treatment protocols currently employ radiosensitizers activated by the electromagnetic radiation of X-rays. Examples of X-ray activated radiosensitizers include, but are not limited to, the following: metronidazole, misonidazole, desmethylmisonidazole, pimonidazole, etanidazole, nimorazole, mitomycin C, RSU 1069, SR 4233, EO9, RB 6145, nicotinamide, 5-bromodeoxyuridine (BUdR), 5-iododeoxyuridine (IUdR), bromodeoxycytidine, fluorodeoxyuridine (FUDR), hydroxyurea, cisplatin, and therapeutically effective analogs and derivatives of the same.

Photodynamic therapy (PDT) of cancers employs visible light as the radiation activator of the sensitizing agent. Examples of photodynamic radiosensitizers include the following, but are not limited to: hematoporphyrin derivatives, Photofrin(r), benzoporphyrin derivatives, NPe6, tin etioporphyrin (SnET2), pheoborbide-a, bacteriochlorophyll-a, naphthalocyanines, phthalocyanines, zinc phthalocyanine, and therapeutically effective analogs and derivatives of the same.

Chemotherapy treatment can employ anti-neoplastic agents including, for example, alkylating agents including: nitrogen mustards, such as mechlorethamine, cyclophosphamide, ifosfamide, melphalan and chlorambucil; nitrosoureas, such as carmustine (BCNU), lomustine (CCNU), and semustine (methyl-CCNU); ethylenimines/methylmelamine such as triethylenemelamine (TEM), triethylene, thiophosphoramido (thiotepa), hexamethylmelamine (HMM, altretamine); alkyl sulfonates such as busulfan; triazines such as dacarbazine (DTIC); antimetabolites including folic acid analogs such as methotrexate and trimetrexate, pyrimidine analogs such as 5-fluorouracil, fluorodeoxyuridine, gemcitabine, cytosine arabinoside (AraC, cytarabine), 5-azacytidine, 2,2'-difluorodeoxycytidine, purine analogs such as 6-

mercaptopurine, 6-thioguanine, azathioprine, 2'-deoxycoformycin (pentostatin), erythrohydroxynonyladenine (EHNA), fludarabine phosphate, and 2-chlorodeoxyadenosine (cladribine, 2-CdA); natural products including antimitotic drugs such as paclitaxel, vinca alkaloids including vinblastine (VLB), vincristine, and

5 vinorelbine, taxotere, estramustine, and estramustine phosphate; piperodophylotoxins such as etoposide and teniposide; antibiotics such as actimomycin D, daunomycin (rubidomycin), doxorubicin, mitoxantrone, idarubicin, bleomycins, plicamycin (mithramycin), mitomycinC, and actinomycin; enzymes such as L-asparaginase; biological response modifiers such as interferon-alpha, IL-2, G-CSF and GM-CSF;

10 miscellaneous agents including platinum coordination complexes such as cisplatin and carboplatin, anthracenediones such as mitoxantrone, substituted urea such as hydroxyurea, methylhydrazine derivatives including N-methylhydrazine (MH) and procarbazine, adrenocortical suppressants such as mitotane (o,p'-DDD) and aminoglutethimide; hormones and antagonists including adrenocorticosteroid antagonists

15 such as prednisone and equivalents, dexamethasone and aminoglutethimide; progestin such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogen such as diethylstilbestrol and ethinyl estradiol equivalents; antiestrogen such as tamoxifen; androgens including testosterone propionate and fluoxymesterone/equivalents; antiandrogens such as flutamide, gonadotropin-releasing

20 hormone analogs and leuprolide; and non-steroidal antiandrogens such as flutamide.

Combination therapy with growth factors can include cytokines, lymphokines, growth factors, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, GM-CSF, thrombopoietin, stem cell factor, and erythropoietin. Other compositions can include known angiopoietins, for example, vascular endothelial growth factor (VEGF). Growth factors include angiogenin, bone morphogenic protein-1, bone morphogenic protein-2, bone morphogenic protein-3, bone morphogenic protein-4, bone morphogenic protein-5, bone morphogenic protein-6, bone morphogenic protein-7, bone morphogenic protein-8, bone morphogenic protein-9, bone morphogenic protein-10, bone morphogenic protein-11, bone morphogenic protein-12, bone morphogenic protein-13, bone morphogenic

protein-14, bone morphogenic protein-15, bone morphogenic protein receptor IA, bone morphogenic protein receptor IB, brain derived neurotrophic factor, ciliary neutrophic factor, ciliary neutrophic factor receptor, cytokine-induced neutrophil chemotactic factor 1, cytokine-induced neutrophil, chemotactic factor 2, cytokine-induced neutrophil chemotactic factor 2, endothelial cell growth factor, endothelin 1, epidermal growth factor, epithelial-derived neutrophil attractant, fibroblast growth factor 4, fibroblast growth factor 5, fibroblast growth factor 6, fibroblast growth factor 7, fibroblast growth factor 8, fibroblast growth factor 8b, fibroblast growth factor 8c, fibroblast growth factor 9, fibroblast growth factor 10, fibroblast growth factor acidic, fibroblast growth factor basic, glial cell line-derived neutrophic factor receptor 1, glial cell line-derived neutrophic factor receptor 2, growth related protein, growth related protein, growth related protein, growth related protein, heparin binding epidermal growth factor, hepatocyte growth factor, hepatocyte growth factor receptor, insulin-like growth factor I, insulin-like growth factor receptor, insulin-like growth factor II, insulin-like growth factor binding protein, keratinocyte growth factor, leukemia inhibitory factor, leukemia inhibitory factor receptor, nerve growth factor nerve growth factor receptor, neurotrophin-3; neurotrophin-4, placenta growth factor, placenta growth factor 2, platelet-derived endothelial cell growth factor, platelet derived growth factor, platelet derived growth factor A chain, platelet derived growth factor AA, platelet derived growth factor AB, platelet derived growth factor B chain, platelet derived growth factor BB, platelet derived growth factor receptor, platelet derived growth factor receptor, pre-B cell growth stimulating factor, stem cell factor, stem cell factor receptor, transforming growth factor, transforming growth factor, transforming growth factor 1, transforming growth factor 1.2, transforming growth factor 2, transforming growth factor 3, transforming growth factor 5, latent transforming growth factor 1, transforming growth factor binding protein I, transforming growth factor binding protein II, transforming growth factor binding protein III, tumor necrosis factor receptor type I, tumor necrosis factor receptor type II, urokinase-type plasminogen activator receptor, vascular endothelial growth factor, and chimeric proteins and biologically or immunologically active fragments thereof.

DIAGNOSTIC USES OF SEQ ID NO: 2 OR 4**(1) ASSAYS FOR DETERMINING SEQ ID NO: 2 OR 4-EXPRESSION STATUS**

Determining the status of SEQ ID NO: 2 or 4 expression patterns in an individual may be used to diagnose cancer and may provide prognostic information useful in

5 defining appropriate therapeutic options. Similarly, the expression status of SEQ ID NO: 2 or 4 may provide information useful for predicting susceptibility to particular disease stages, progression, and/or tumor aggressiveness. The invention provides methods and assays for determining SEQ ID NO: 2 or 4 expression status and diagnosing cancers that express SEQ ID NO: 2 or 4.

10 In one aspect, the invention provides assays useful in determining the presence of cancer in an individual, comprising detecting a significant increase in SEQ ID NO: 2 or 4-encoding mRNA or protein expression in a test cell or tissue or fluid sample relative to expression levels in the corresponding normal cell or tissue. In one embodiment, the presence of SEQ ID NO: 2 or 4 encoding mRNA is evaluated in tissue samples of a

15 lymphoma. The presence of significant SEQ ID NO: 2 or 4 expression may be useful to indicate whether the lymphoma is susceptible to SEQ ID NO: 2 or 4 immunotargeting. In a related embodiment, SEQ ID NO: 2 or 4 expression status may be determined at the protein level rather than at the nucleic acid level. For example, such a method or assay would comprise determining the level of SEQ ID NO: 2 or 4 expressed by cells in a test 20 tissue sample and comparing the level so determined to the level of SEQ ID NO: 2 or 4 expressed in a corresponding normal sample. In one embodiment, the presence of SEQ ID NO: 2 or 4 is evaluated, for example, using immunohistochemical methods. Anti-SEQ ID NO: 2 or 4 antibodies capable of detecting SEQ ID NO: 2 or 4 expression may be used in a variety of assay formats well known in the art for this purpose.

25 Peripheral blood may be conveniently assayed for the presence of cancer cells, including lymphomas and leukemias, using RT-PCR to detect SEQ ID NO: 2 or 4 expression. The presence of RT-PCR amplifiable SEQ ID NO: 2 or 4-encoding mRNA provides an indication of the presence of one of these types of cancer. A sensitive assay for detecting and characterizing carcinoma cells in blood may be used (Racila, *et al.*, 30 *Proc. Natl. Acad. Sci. USA* 95: 4589-4594 (1998)). This assay combines immunomagnetic enrichment with multiparameter flow cytometric and

immunohistochemical analyses, and is highly sensitive for the detection of cancer cells in blood, reportedly capable of detecting one epithelial cell in 1 ml of peripheral blood.

A related aspect of the invention is directed to predicting susceptibility to developing cancer in an individual. In one embodiment, a method for predicting 5 susceptibility to cancer comprises detecting SEQ ID NO: 2 or 4-encoding mRNA or SEQ ID NO: 2 or 4 in a tissue sample, its presence indicating susceptibility to cancer, wherein the degree of SEQ ID NO: 2 or 4-encoding mRNA expression present is proportional to the degree of susceptibility.

Yet another related aspect of the invention is directed to methods for assessment 10 of tumor aggressiveness (Orlandi, *et al.*, *Cancer Res.* 62:567 (2002)). In one embodiment, a method for gauging aggressiveness of a tumor comprises determining the level of SEQ ID NO: 2 or 4-encoding mRNA or SEQ ID NO: 2 or 4 protein expressed by cells in a sample of the tumor, comparing the level so determined to the level of SEQ ID 15 NO: 2 or 4-encoding mRNA or SEQ ID NO: 2 or 4 protein expressed in a corresponding normal tissue taken from the same individual or a normal tissue reference sample, wherein the degree of SEQ ID NO: 2 or 4-encoding mRNA or SEQ ID NO: 2 or 4 protein expression in the tumor sample relative to the normal sample indicates the degree of aggressiveness.

Methods for detecting and quantifying the expression of SEQ ID NO: 2 or 4-encoding mRNA or protein are described herein and use standard nucleic acid and 20 protein detection and quantification technologies well known in the art. Standard methods for the detection and quantification of SEQ ID NO: 2 or 4-encoding mRNA include *in situ* hybridization using labeled SEQ ID NO: 2 or 4-encoding riboprobes (Gemou-Engesaeth, *et al.*, *Pediatrics*, 109:E24-E32 (2002)), Northern blot and related 25 techniques using SEQ ID NO: 2 or 4-encoding polynucleotide probes (Kunzli, *et al.*, *Cancer* 94:228 (2002)), RT-PCR analysis using primers specific for SEQ ID NO: 2 or 4-encoding polynucleotides (Angchaiskisiri, *et al.*, *Blood* 99:130 (2002)), and other amplification type detection methods, such as, for example, branched DNA (Jardi, *et al.*, *J. Viral Hepat.* 8:465-471 (2001)), SISBA, TMA (Kimura, *et al.*, *J. Clin. Microbiol.* 30 40:439-445 (2002)), and microarray products of a variety of sorts, such as oligos, cDNAs, and monoclonal antibodies. In a specific embodiment, real-time RT-PCR may

be used to detect and quantify SEQ ID NO: 2 or 4-encoding mRNA expression (Simpson, *et al.*, *Molec. Vision* 6:178-183 (2000)). Standard methods for the detection and quantification of protein may be used for this purpose. In a specific embodiment, polyclonal or monoclonal antibodies specifically reactive with the wild-type SEQ ID NO:

5 2 or 4 may be used in an immunohistochemical assay of biopsied tissue (Ristimaki, *et al.*, *Cancer Res.* 62:632 (2002)).

(2) MEDICAL IMAGING

Anti-SEQ ID NO: 2 or 4 antibodies and fragments thereof are useful in medical imaging of sites expressing SEQ ID NO: 2 or 4. Such methods involve chemical attachment of a labeling or imaging agent, such as a radioisotope, which include ⁶⁷Cu, ⁹⁰Y, ¹²⁵I, ¹³¹I, ¹⁸⁶Re, ¹⁸⁸Re, ²¹¹At, ²¹²Bi, administration of the labeled antibody and fragment to a subject in a pharmaceutically acceptable carrier, and imaging the labeled antibody and fragment *in vivo* at the target site. Radiolabelled anti-SEQ ID NO: 2 or 4 antibodies or fragments thereof may be particularly useful in *in vivo* imaging of SEQ ID NO: 2 or 4 expressing cancers, such as lymphomas or leukemias. Such antibodies may provide highly sensitive methods for detecting metastasis of SEQ ID NO: 2 or 4-expressing cancers.

Upon consideration of the present disclosure, one of skill in the art will appreciate that many other embodiments and variations may be made in the scope of the present invention. Accordingly, it is intended that the broader aspects of the present invention not be limited to the disclosure of the following examples.

25

EXAMPLE 1

CELL LINES OF LYMPHOMA AND LEUKEMIA ORIGIN EXPRESS HIGH LEVELS OF
SEQ ID NO: 2 or 4-ENCODING mRNA

Expression of SEQ ID NO: 2 or 4 was determined in various lymphoid and myeloid cell lines. Poly-A messenger RNA was isolated from the cell lines listed in Table 1 and subjected to quantitative, real-time PCR analysis (Simpson, *et al.*, *Molec. Vision* 6: 178-183 (2000)) to determine the relative copy number of SEQ ID NO: 2 or 4-

encoding mRNA expressed per cell in each line. Elongation factor 1 mRNA expression was used as a positive control and normalization factors in all samples.

All assays were performed in duplicate with the resulting values averaged and expressed as “-“ for samples with no detectable SEQ ID NO: 2 or 4-encoding mRNA in that sample to “++” for samples with the highest copy number for SEQ ID NO: 2 or 4-encoding mRNA. The following quantitation scale for the real-time PCR experiments was used: “-“ = 0 copies/cell; “+“ = approximately 1-10 copies/cell; “++“ = approximately 11-50 copies/cell; and “+++“ = approximately >50 copies/cell. The results are indicated in Tables 1A and 1B.

10

Table 1A (SEQ ID NO: 2)

Cell Line	SEQ ID NO: 2-encoding mRNA Expression
B Diffuse Mixed Cell Lymphoma (HT)	+
Non-Hodgkin's Lymphoma (RL)	+
Burkitt's Lymphoma (CA-46)	+
Acute Myelogenous Leukemia (KG-1)	-
T cell Leukemia (Molt-4)	-

Table 1B (SEQ ID NO: 4)

Cell Line	SEQ ID NO: 4-encoding mRNA Expression
Acute Myeloid Leukemia (AML565)	-
Acute Monocytic Leukemia (AML193)	++
Non-Hodgkin's Lymphoma (RL)	++
Burkitt's Lymphoma (CA-46)	++
Acute Myelogenous Leukemia (KG-1)	++
T cell Leukemia (Molt-4)	++
Activated CD8	-

Activated CD4	++
Activated Monocytes	++

EXAMPLE 2**SEQ ID NO: 2 OR 4-ENCODING mRNA IS HIGHLY EXPRESSED IN PATIENT TISSUES**

5 Expression of SEQ ID NO: 2 or 4 was determined in various healthy and tumor tissues (Table 2). Poly-A mRNA was isolated from frozen patient tissue samples obtained from the Cooperative Human Tissue Network (CHTN, National Cancer Institute). All other RNAs were purchased from Clontech (Palo Alto, CA) and Ambion (Austin, TX). Poly-A⁺ mRNA was subjected to quantitative, real-time PCR analysis, as
10 described in Example 1, to determine the relative expression of SEQ ID NO: 2 or 4-encoding mRNA in the sample. All assays were performed in duplicate with the resulting values averaged and expressed as “-“ for samples with no detectable SEQ ID NO: 2 or 4-encoding mRNA in that sample to “+++” for samples with the highest copy number for SEQ ID NO: 2 or 4-encoding mRNA. The following quantitation scale for the
15 real-time PCR experiments was used: “-“ = 0 copies/cell; “+“ = approximately 1-10 copies/cell; “++“ = approximately 11-50 copies/cell; and “+++“ = approximately >50 copies/cell. The results are indicated in Tables 2A and 2B.

Table 2A (SEQ ID NO: 2)

Patient Tissue	SEQ ID NO: 2-encoding mRNA Expression
Anaplastic Large T cell Lymphoma L5664	++
Large Follicular Lymphoma L25301	++
Follicular Lymphoma grade II L5348	++
Diffuse Large B Cell Lymphoma L6879	++
Spleen	++
Colon	++
Lung	+

Liver	-
Brain	-
Kidney	-
Stomach	-
Pancreas	-
Breast	-
Ovary	-
Heart	-
Placenta	-
Prostate	-

Table 2B (SEQ ID NO: 4)

Patient Tissue	SEQ ID NO: 4-encoding mRNA Expression
Spleen	++
Placenta	-
Ovary	-
Pancreas	-
Stomach	-
Kidney	-
Liver	-
Brain	-
Lung	-
Colon	-
Breast	-
Prostate	-
Bladder	-
Heart	-

EXAMPLE 3**PRODUCTION OF SEQ ID NO: 2 OR 4-SPECIFIC ANTIBODIES**

Cells expressing SEQ ID NO: 2 or 4 are identified using antibodies to SEQ ID NO: 2 or 4. Polyclonal antibodies are produced by DNA vaccination or by injection of peptide antigens into rabbits or other hosts. An animal, such as a rabbit, is immunized with a peptide from the extracellular region of SEQ ID NO: 2 or 4 conjugated to a carrier protein, such as BSA (bovine serum albumin) or KLH (keyhole limpet hemocyanin). The rabbit is initially immunized with conjugated peptide in complete Freund's adjuvant, followed by a booster shot every two weeks with injections of conjugated peptide in incomplete Freund's adjuvant. Anti-SEQ ID NO: 2 or 4 antibody is affinity purified from rabbit serum using SEQ ID NO: 2 or 4 peptide coupled to Affi-Gel 10 (Bio-Rad), and stored in phosphate-buffered saline with 0.1% sodium azide. To determine that the polyclonal antibodies are SEQ ID NO: 2 or 4-specific, an expression vector encoding SEQ ID NO: 2 or 4 is introduced into mammalian cells. Western blot analysis of protein extracts of non-transfected cells and the SEQ ID NO: 2 or 4-containing cells is performed using the polyclonal antibody sample as the primary antibody and a horseradish peroxidase-labeled anti-rabbit antibody as the secondary antibody. Detection of a band in the SEQ ID NO: 2 or 4-containing cells and lack thereof in the control cells indicates that the polyclonal antibodies are specific for SEQ ID NO: 2 or 4.

Monoclonal antibodies are produced by injecting mice with a SEQ ID NO: 2 or 4 peptide, with or without adjuvant. Subsequently, the mouse is boosted every 2 weeks until an appropriate immune response has been identified (typically 1-6 months), at which point the spleen is removed. The spleen is minced to release splenocytes, which are fused (in the presence of polyethylene glycol) with murine myeloma cells. The resulting cells (hybridomas) are grown in culture and selected for antibody production by clonal selection. The antibodies are secreted into the culture supernatant, facilitating the screening process, such as screening by an enzyme-linked immunosorbent assay (ELISA). Alternatively, humanized monoclonal antibodies are produced either by engineering a chimeric murine/human monoclonal antibody in which the murine-specific antibody regions are replaced by the human counterparts and produced in mammalian cells, or by using transgenic "knock out" mice in which the native antibody genes have

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been replaced by human antibody genes and immunizing the transgenic mice as described above.

EXAMPLE 4

5

IN VITRO ANTIBODY-DEPENDENT CYTOTOXICITY ASSAY

The ability of a SEQ ID NO: 2 or 4-specific antibody to induce antibody-dependent cell-mediated cytotoxicity (ADCC) is determined *in vitro*. ADCC is performed using the CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega; Madison, WI) (Hornick *et al.*, *Blood* 89:4437-4447, (1997)) as well as effector and target cells.

10 Peripheral blood mononuclear cells (PBMC) or neutrophilic polymorphonuclear leukocytes (PMN) are two examples of effector cells that can be used in this assay. PBMC are isolated from healthy human donors by Ficoll-Paque gradient centrifugation, and PMN are purified by centrifugation through a discontinuous percoll gradient (70% and 62%) followed by hypotonic lysis to remove residual erythrocytes. RA1 B cell

15 lymphoma cells (for example) are used as target cells.

RA1 cells are suspended in RPMI 1640 medium supplemented with 2% fetal bovine serum and plated in 96-well V-bottom microtiter plates at 2×10^4 cells/well. SEQ ID NO: 2 or 4-specific antibody is added in triplicate to individual wells at 1 $\mu\text{g}/\text{ml}$, and effector cells are added at various effector:target cell ratios (12.5:1 to 50:1). The plates are incubated for 4 hours at 37°C. The supernatants are then harvested, lactate dehydrogenase release determined, and percent specific lysis calculated using the manufacturer's protocols.

EXAMPLE 5

25

TOXIN-CONJUGATED SEO ID NO: 2 OR 4-SPECIFIC ANTIBODIES

Antibodies to SEQ ID NO: 2 or 4 are conjugated to toxins and the effect of such conjugates in animal models of cancer is evaluated. Chemotherapeutic agents, such as calicheamycin and carboplatin, or toxic peptides, such as ricin toxin, are used in this approach. Antibody-toxin conjugates are used to target cytotoxic agents specifically to cells bearing the antigen. The antibody-toxin binds to these antigen-bearing cells, becomes internalized by receptor-mediated endocytosis, and subsequently destroys the

targeted cell. In this case, the antibody-toxin conjugate targets SEQ ID NO: 2 or 4-expressing cells, such as B cell lymphomas, and deliver the cytotoxic agent to the tumor resulting in the death of the tumor cells.

One such example of a toxin that may be conjugated to an antibody is carboplatin.

- 5 The mechanism by which this toxin is conjugated to antibodies is described in Ota *et al.*, *Asia-Oceania J. Obstet. Gynaecol.* 19: 449-457 (1993). The cytotoxicity of carboplatin-conjugated SEQ ID NO: 2 or 4-specific antibodies is evaluated *in vitro*, for example, by incubating SEQ ID NO: 2 or 4-expressing target cells (such as the RA1 B cell lymphoma cell line) with various concentrations of conjugated antibody, medium alone, carboplatin alone, or antibody alone. The antibody-toxin conjugate specifically targets and kills cells bearing the SEQ ID NO: 2 or 4 antigen, whereas, cells not bearing the antigen, or cells treated with medium alone, carboplatin alone, or antibody alone, show no cytotoxicity.

- 10 The antitumor efficacy of carboplatin-conjugated SEQ ID NO: 2 or 4-specific antibodies is demonstrated in *in vivo* murine tumor models. Five to six week old, athymic nude mice are engrafted with tumors subcutaneously or through intravenous injection. Mice are treated with the SEQ ID NO: 2 or 4-carboplatin conjugate or with a non-specific antibody-carboplatin conjugate. Tumor xenografts in the mouse bearing the SEQ ID NO: 2 or 4 antigen are targeted and bound to by the SEQ ID NO: 2 or 4-carboplatin conjugate. This results in tumor cell killing as evidenced by tumor necrosis, tumor shrinkage, and increased survival of the treated mice.

- 15 Other toxins are conjugated to CD84Hy1-specific antibodies using methods known in the art. An example of a toxin conjugated antibody in human clinical trials is CMA-676, an antibody to the CD33 antigen in AML which is conjugated with calicheamicin toxin (Larson, *Semin. Hematol.* 38(Suppl 6):24-31 (2001)).

25

EXAMPLE 6

RADIO-IMMUNOTHERAPY USING SEQ ID NO: 2 OR 4-SPECIFIC ANTIBODIES

- 20 Animal models are used to assess the effect of antibodies specific to SEQ ID NO: 2 or 4 as vectors in the delivery of radionuclides in radio-immunotherapy to treat lymphoma, hematological malignancies, and solid tumors. Human tumors are propagated in 5-6 week old athymic nude mice by injecting a carcinoma cell line or

tumor cells subcutaneously. Tumor-bearing animals are injected intravenously with radio-labeled anti-SEQ ID NO: 2 or 4 antibody (labeled with 30-40 µCi of ^{131}I , for example) (Behr, *et al.*, *Int. J. Cancer* 77: 787-795 (1988)). Tumor size is measured before injection and on a regular basis (i.e. weekly) after injection and compared to 5 tumors in mice that have not received treatment. Anti-tumor efficacy is calculated by correlating the calculated mean tumor doses and the extent of induced growth retardation. To check tumor and organ histology, animals are sacrificed by cervical dislocation and autopsied. Organs are fixed in 10% formalin, embedded in paraffin, and thin sectioned. The sections are stained with hematoxylin-eosin.

10

EXAMPLE 7

IMMUNOTHERAPY USING SEQ ID NO: 2 OR 4-SPECIFIC ANTIBODIES

15 Animal models are used to evaluate the effect of CD84Hy1-specific antibodies as targets for antibody-based immunotherapy using monoclonal antibodies. Human myeloma cells are injected into the tail vein of 5-6 week old nude mice whose natural killer cells have been eradicated. To evaluate the ability of SEQ ID NO: 2 or 4-specific 20 antibodies in preventing tumor growth, mice receive an intraperitoneal injection with SEQ ID NO: 2 or 4-specific antibodies either 1 or 15 days after tumor inoculation followed by either a daily dose of 20 µg or 100 µg once or twice a week, respectively (Ozaki, *et al.*, *Blood* 90:3179-3186 (1997)). Levels of human IgG (from the immune reaction caused by the human tumor cells) are measured in the murine sera by ELISA.

25 The effect of SEQ ID NO: 2 or 4-specific antibodies on the proliferation of myeloma cells is examined *in vitro* using a ^3H -thymidine incorporation assay (Ozaki *et al.*, *supra*). Cells are cultured in 96-well plates at 1×10^5 cells/ml in 100 µl/well and incubated with various amounts of SEQ ID NO: 2 or 4 antibody or control IgG (up to 100 µg/ml) for 24 h. Cells are incubated with 0.5 µCi ^3H -thymidine (New England Nuclear, Boston, MA) for 18 h and harvested onto glass filters using an automatic cell harvester 30 (Packard, Meriden, CT). The incorporated radioactivity is measured using a liquid scintillation counter.

The cytotoxicity of the anti-SEQ ID NO: 2 or 4 monoclonal antibody is examined by the effect of complements on myeloma cells using a ^{51}Cr -release assay (Ozaki *et al.*, *supra*). Myeloma cells are labeled with 0.1 mCi ^{51}Cr -sodium chromate at 37°C for 1 h. ^{51}Cr -labeled cells are incubated with various concentrations of anti-SEQ ID NO: 2 or 4

5 monoclonal antibody or control IgG on ice for 30 min. Unbound antibody is removed by washing with medium. Cells are distributed into 96-well plates and incubated with serial dilutions of baby rabbit complement at 37°C for 2 h. The supernatants are harvested from each well and the amount of ^{51}Cr released is measured using a gamma counter. Spontaneous release of ^{51}Cr is measured by incubating cells with medium alone, whereas

10 maximum ^{51}Cr release is measured by treating cells with 1% NP-40 to disrupt the plasma membrane. Percent cytotoxicity is measured by dividing the difference of experimental and spontaneous ^{51}Cr release by the difference of maximum and spontaneous ^{51}Cr release.

Antibody-dependent cell-mediated cytotoxicity (ADCC) for the anti-SEQ ID NO: 2 or 4 monoclonal antibody is measured using a standard 4 h ^{51}Cr -release assay (Ozaki *et al.*, *supra*). Splenic mononuclear cells from SCID mice are used as effector cells and cultured with or without recombinant interleukin-2 (for example) for 6 days. ^{51}Cr -labeled target myeloma cells (1×10^4 cells) are placed in 96-well plates with various concentrations of anti-SEQ ID NO: 2 or 4 monoclonal antibody or control IgG. Effector cells are added to the wells at various effector to target ratios (12.5:1 to 50:1). After 4 h, culture supernatants are removed and counted in a gamma counter. The percentage of cell lysis is determined as above.

EXAMPLE 8

25 **SEQ ID NO: 2 OR 4-SPECIFIC ANTIBODIES AS IMMUNOSUPPRESSANTS**

Animal models are used to assess the effect of SEQ ID NO: 2 or 4-specific antibodies block signaling through the SEQ ID NO: 2 or 4 receptor to suppress autoimmune diseases, such as arthritis or other inflammatory conditions, or rejection of organ transplants. Immunosuppression is tested by injecting mice with horse red blood cells (HRBCs) and assaying for the levels of HRBC-specific antibodies (Yang, *et al.*, *Int. Immunopharm.* 2:389-397 (2002)). Animals are divided into five groups, three of which

are injected with anti-SEQ ID NO: 2 or 4 antibodies for 10 days, and 2 of which receive no treatment. Two of the experimental groups and one control group are injected with either Earle's balanced salt solution (EBSS) containing $5-10 \times 10^7$ HRBCs or EBSS alone. Anti-SEQ ID NO: 2 or 4 antibody treatment is continued for one group while the other groups receive no antibody treatment. After 6 days, all animals are bled by retro-orbital puncture, followed by cervical dislocation and spleen removal. Splenocyte suspensions are prepared and the serum is removed by centrifugation for analysis.

5 Immunosuppression is measured by the number of B cells producing HRBC-specific antibodies. The Ig isotype (for example, IgM, IgG1, IgG2, etc.) is determined
 10 using the IsoDetect™ Isotyping kit (Stratagene, La Jolla, CA). Once the Ig isotype is known, murine antibodies against HRBCs are measured using an ELISA procedure. 96-well plates are coated with HRBCs and incubated with the anti-HRBC antibody-containing sera isolated from the animals. The plates are incubated with alkaline phosphatase-labeled secondary antibodies and color development is measured on a
 15 microplate reader (SPECTRAmax 250, Molecular Devices) at 405 nm using *p*-nitrophenyl phosphate as a substrate.

20 Lymphocyte proliferation is measured in response to the T and B cell activators concanavalin A and lipopolysaccharide, respectively (Jiang, *et al.*, *J. Immunol.* 154:3138-3146 (1995)). Mice are randomly divided into 2 groups, 1 receiving anti-SEQ ID NO: 2 or 4 antibody therapy for 7 days and 1 as a control. At the end of the treatment, the animals are sacrificed by cervical dislocation, the spleens are removed, and splenocyte suspensions are prepared as above. For the *ex vivo* test, the same number of splenocytes are used, whereas for the *in vivo* test, the anti-SEQ ID NO: 2 or 4 antibody is added to the medium at the beginning of the experiment. Cell proliferation is also assayed using the
 25 ^3H -thymidine incorporation assay described above (Ozaki, *et al.*, *Blood* 90: 3179 (1997)).

EXAMPLE 9

CYTOKINE SECRETION IN RESPONSE TO SEQ ID NO: 2 OR 4 PEPTIDE FRAGMENTS

Assays are carried out to assess activity of fragments of the SEQ ID NO: 2 or 4 protein, such as the Ig domain, to stimulate cytokine secretion and to stimulate immune responses in NK cells, B cells, T cells, and myeloid cells. Such immune responses can be

used to stimulate the immune system to recognize and/or mediate tumor cell killing or suppression of growth. Similarly, this immune stimulation can be used to target bacterial or viral infections. Alternatively, fragments of the SEQ ID NO: 2 or 4 that block activation through the SEQ ID NO: 2 or 4 receptor may be used to block immune 5 stimulation in natural killer (NK), B, T, and myeloid cells.

Fusion proteins containing fragments of the SEQ ID NO: 2 or 4, such as the Ig domain (SEQ ID NO: 2 or 4-Ig), are made by inserting a CD33 leader peptide, followed by a SEQ ID NO: 2 or 4 domain fused to the Fc region of human IgG1 into a mammalian expression vector, which is stably transfected into NS-1 cells, for example. The fusion 10 proteins are secreted into the culture supernatant, which is harvested for use in cytokine assays, such as interferon- γ (IFN- γ) secretion assays (Martin, *et al.*, *J. Immunol.* 167:3668-3676 (2001)).

PBMCs are activated with a suboptimal concentration of soluble CD3 and various concentrations of purified, soluble anti-SEQ ID NO: 2 or 4 monoclonal antibody or 15 control IgG. For SEQ ID NO: 2 or 4-Ig cytokine assays, anti-human Fc Ig at 5 or 20 μ g/ml is bound to 96-well plates and incubated overnight at 4°C. Excess antibody is removed and either SEQ ID NO: 2 or 4-Ig or control Ig is added at 20-50 μ g/ml and incubated for 4 h at room temperature. The plate is washed to remove excess fusion protein before adding cells and anti-CD3 to various concentrations. Supernatants are 20 collected after 48 h of culture and IFN- γ levels are measured by sandwich ELISA, using primary and biotinylated secondary anti-human IFN- γ antibodies as recommended by the manufacturer.

EXAMPLE 10

25 **DIAGNOSTIC METHODS USING SEQ ID NO: 2 OR 4-SPECIFIC ANTIBODIES TO DETECT
SEQ ID NO: 2 OR 4 EXPRESSION**

Expression of SEQ ID NO: 2 or 4 in tissue samples (normal or diseased) is detected using anti-SEQ ID NO: 2 or 4 antibodies. Samples are prepared for 30 immunohistochemical (IHC) analysis by fixing the tissue in 10% formalin embedding in paraffin, and sectioning using standard techniques. Sections are stained using the SEQ ID NO: 2 or 4-specific antibody followed by incubation with a secondary horse radish

peroxidase (HRP)-conjugated antibody and visualized by the product of the HRP enzymatic reaction.

Expression of SEQ ID NO: 2 or 4 on the surface of cells within a blood sample is detected by flow cytometry. Peripheral blood mononuclear cells (PBMC) are isolated 5 from a blood sample using standard techniques. The cells are washed with ice-cold PBS and incubated on ice with the SEQ ID NO: 2 or 4-specific polyclonal antibody for 30 min. The cells are gently pelleted, washed with PBS, and incubated with a fluorescent anti-rabbit antibody for 30 min. on ice. After the incubation, the cells are gently pelleted, washed with ice cold PBS, and resuspended in PBS containing 0.1% sodium azide and 10 stored on ice until analysis. Samples are analyzed using a FACScalibur flow cytometer (Becton Dickinson) and CELLQuest software (Becton Dickinson). Instrument setting are determined using FACS-Brite calibration beads (Becton-Dickinson).

Tumors expressing SEQ ID NO: 2 or 4 is imaged using SEQ ID NO: 2 or 4-specific antibodies conjugated to a radionuclide, such as ^{123}I , and injected into the patient 15 for targeting to the tumor followed by X-ray or magnetic resonance imaging.

EXAMPLE 11

TUMOR IMAGING USING SEQ ID NO: 2 OR 4-SPECIFIC ANTIBODIES

SEQ ID NO: 2 or 4-specific antibodies are used for imaging SEQ ID NO: 2 or 4-expressing cells *in vivo*. Six-week-old athymic nude mice are irradiated with 400 rads 20 from a cesium source. Three days later the irradiated mice are inoculated with 4×10^7 RA1 cells and 4×10^6 human fetal lung fibroblast feeder cells subcutaneously in the thigh. When the tumors reach approximately 1 cm in diameter, the mice are injected 25 intravenously with an inoculum containing 100 $\mu\text{Ci}/10 \mu\text{g}$ of ^{131}I -labeled SEQ ID NO: 2 or 4-specific antibody. At 1, 3, and 5 days postinjection, the mice are anesthetized with a subcutaneous injection of 0.8 mg sodium pentobarbital. The immobilized mice are then imaged in a prone position with a Spectrum 91 camera equipped with a pinhole 30 collimator (Raytheon Medical Systems; Melrose Park, IL) set to record 5,000 to 10,000 counts using the Nuclear MAX Plus image analysis software package (MEDX Inc.; Wood Dale, IL) (Hornick, *et al.*, *Blood* 89:4437-4447 (1997)).

WE CLAIM:

1. An isolated polynucleotide comprising a nucleotide sequence consisting of SEQ ID NO: 3 or the mature protein coding portion thereof.
- 5 2. An isolated polynucleotide encoding a polypeptide with biological activity, said polynucleotide having greater than about 99% sequence identity with the polynucleotide of claim 1.
- 10 3. The polynucleotide of claim 1 which is a RNA sequence.
4. An isolated polynucleotide which comprises the full complement of the polynucleotide of claim 1.
- 15 5. An isolated polypeptide comprising an amino acid sequence which is at least 99% identical to the amino acid sequence consisting of SEQ ID NO: 4.
6. A polynucleotide encoding a polypeptide according to claim 5.
- 20 7. A nucleic acid array comprising the polynucleotide of claim 1 attached to a surface.
8. A pharmaceutical composition comprising an anti-SEQ ID NO: 2 or 4 antibody specific for cells that cause a cancer selected from the group consisting of T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage, wherein said antibody specifically binds to a polypeptide having an amino acid sequence of SEQ ID. NO: 2 or 4
- 30 9. The pharmaceutical composition of claim 1, wherein said antibody is a monoclonal anti-SEQ ID NO: 2 or 4 antibody or fragment thereof.

10. The pharmaceutical composition of claim 1, wherein said antibody is administered in an amount effective to kill or inhibit the growth of cells that cause a cancer selected from the group consisting T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-
5 Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage.

11. A method of targeting SEQ ID NO: 2 or 4 protein on cells that cause a cancer selected from the group consisting of T-cell lymphoma, T-cell leukemia, multiple
10 myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage, comprising the step of administering a composition to said cells in an amount effective to target said SEQ ID NO: 2 or 4-expressing cells, wherein said composition is an anti-SEQ ID NO: 2 or 4 antibody that specifically binds to a
15 polypeptide having an amino acid sequence of SEQ ID NO: 2 or 4.

12. A method of killing or inhibiting the growth of SEQ ID NO: 2 or 4-expressing cells that cause a cancer selected from the group consisting of T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage, comprising the step of administering a composition to said cells in an amount effective to kill or inhibit the growth of said cancer cells, wherein said composition is an anti-SEQ ID NO: 2 or 4 antibody that specifically binds to a polypeptide having an amino acid sequence of SEQ ID. NO: 2 or
25 4.

13. A method of killing or inhibiting the growth of SEQ ID NO: 2 or 4-expressing cells that cause a cancer selected from the group consisting of T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage, comprising the step of administering a

vaccine to said cells in an amount effective to kill or inhibit the growth of said cancer cells, wherein said vaccine comprises a polypeptide having an amino acid sequence of SEQ ID NO: 2 or 4, or immunogenic fragment thereof.

5 14. A method of killing or inhibiting the growth of SEQ ID NO: 2 or 4-expressing cells that cause a cancer selected from the group consisting of T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage, comprising the step of administering a
10 composition to said cells in an amount effective to kill or inhibit the growth of said cancer cells, wherein said composition comprises a nucleic acid encoding SEQ ID NO: 2 or 4, or immunogenic fragment thereof, within a recombinant vector.

15 15. A method of killing or inhibiting the growth of SEQ ID NO: 2 or 4-expressing cells that cause a cancer selected from the group consisting of T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage, comprising the step of administering a composition to said cells in an amount effective to kill or inhibit the growth of said
20 cancer cells, wherein said composition comprises an antigen-presenting cell comprising a nucleic acid encoding SEQ ID NO: 2 or 4, or immunogenic fragment thereof, within a recombinant vector.

25 16. The method according to claims 4, 5, 6, 7, or 8, wherein said cells are contacted with as second therapeutic agent.

17. The method according to claim 4 or 5, wherein said anti-SEQ ID NO: 2 or 4 antibody composition is administered in an amount effective to achieve a dosage range from about 0.1 to about 10 mg/kg body weight.

18. The method according to claims 4, 5, 6, 7, or 8, 4, wherein said pharmaceutical composition is administered in a sterile preparation together with a pharmaceutically acceptable carrier therefore.

5 19. A method of diagnosing cancer selected from the group consisting of T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage comprising the steps of:
detecting or measuring the expression of SEQ ID NO: 2 or 4 protein on a
10 cell; and
comparing said expression to a standard indicative of cancer.

20. The method according to claim 12, wherein said expression is SEQ ID NO: 2 or 4-encoding mRNA expression.

15 21. The method according to claim 12, wherein said expression is detected or measured using anti-SEQ ID NO: 2 or 4 antibodies.

20 22. Use of an anti- SEQ ID NO: 2 or 4 antibody in preparation of a medicament for killing or inhibiting the growth of SEQ ID NO: 2 or 4-expressing cells that cause a cancer selected from the group consisting of T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage, wherein said antibody specifically binds to a
25 polypeptide having the amino acid sequence of SEQ ID NO: 2 or 4.

23. Use of a polypeptide having an amino acid sequence of SEQ ID NO: 2 or 4 in preparation of a vaccine for killing or inhibiting the growth of SEQ ID NO: 2 or 4-expressing cells that cause a cancer selected from the group consisting of T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell

lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage.

24. Use of a nucleic acid encoding SEQ ID NO: 2 or 4 or immunogenic fragment thereof, within a recombinant vector, in preparation of a medicament for killing or inhibiting the growth of SEQ ID NO: 2 or 4-expressing cells that cause a cancer selected from the group consisting of T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage.

25. Use of an antigen-presenting cell comprising a nucleic acid encoding SEQ ID NO: 2 or 4 or immunogenic fragment thereof, within a recombinant vector, in preparation of a medicament for killing or inhibiting the growth of SEQ ID NO: 2 or 4-expressing cells that cause a cancer selected from the group consisting of T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage.

METHODS OF IMMUNOTHERAPY AND DIAGNOSIS

ABSTRACT

Certain cells, including types of cancer cells such as T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage, are capable of expressing SEQ ID NO: 2 or 4-encoding RNA. Immunotargeting using SEQ ID NO: 2 or 4 polypeptides, nucleic acids encoding for SEQ ID NO: 2 or 4 polypeptides and anti-SEQ ID NO: 2 or 4 antibodies provides a method of killing or inhibiting that growth of cancer cells that express the SEQ ID NO: 2 or 4 protein. Methods of immunotherapy and diagnosis of disorders associated with SEQ ID NO: 2 or 4 protein-expressing cells, such as T-cell lymphoma, T-cell leukemia, multiple myeloma, and chronic myeloid leukemia, B cell lymphoma of mature B cell lineage, non-Hodgkin's lymphoma of mature B-cell lineage, and Burkitt's lymphoma of mature B cell lineage, are described.

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Docket No.: HYS-67

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As [a] below named inventor(s), I/we hereby declare that:

Peter C.R. Emtage, Y. Tom Tang, Zhiwei Wang, Radoje T. Drmanac

My/our residence, post office address and citizenship is/are as stated below next to my/our name(s).

I/we believe I/we am/are an/the original, first and sole/joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: **METHODS OF IMMUNOTHERAPY AND DIAGNOSIS**, the specification of which

X is attached hereto.

— was filed on [date] as Application Serial Number [] and was amended on [date].

I/We hereby state that I/we have reviewed and understand the contents of the above-identified specification, including the claims as amended by any amendment referred to above.

I/We acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I/We hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate, listed below and so identified, and I/we have also identified below any foreign application for patent or inventor's certificate on this invention filed by me or my legal representatives or assigns and having a filing date before that of the application on which priority is claimed:

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED - YES OR NO

I/We hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I/we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

SERIAL NUMBER	FILING DATE	STATUS
10/128,558	April 22, 2002	Pending
60/339,453	December 11, 2001	Pending

I/We hereby declare that all statements made herein of my/our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I/We hereby appoint the following attorneys and agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith and request that all correspondence and telephone calls with respect to this application be directed to Elena Quertermous, HYSEQ, INC., 670 Almanor Avenue, Sunnyvale, CA 94085, Telephone No. (408) 524-8100:

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HYS-67 Sequence Listing
SEQUENCE LISTING

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aat tgg aat aaa cgg aag aag cga caa gtt cct tac ctc cga gtt acc Asn Trp Asn Lys Arg Lys Arg Gln Val Pro Tyr Leu Arg Val Thr	664
60 65 70 75	
gtc atg ccc ttg ctg act ttg cca caa acc aga caa aga gcc aaa aat Val Met Pro Leu Leu Thr Leu Pro Gln Thr Arg Gln Arg Ala Lys Asn	712
80 85 90	
att tat gac atc ttg cct tgg cga cag gaa gac ctg ggg aga cat gag Ile Tyr Asp Ile Leu Pro Trp Arg Gln Glu Asp Leu Gly Arg His Glu	760
95 100 105	
tcg agg agt atg cgc att ttc agt act gag agc ctc ctc tcc aga aat Ser Arg Ser Met Arg Ile Phe Ser Thr Glu Ser Leu Leu Ser Arg Asn	808
110 115 120	
tct gag agc ccg gag cat gtg ccc tcc caa gca ggc aat gcc ttc cag Ser Glu Ser Pro Glu His Val Pro Ser Gln Ala Gly Asn Ala Phe Gln	856
125 130 135	
gag cat aca gcc cac atc cat gcc aca gag tac gcg gtg ggt atc tat Glu His Thr Ala His Ile His Ala Thr Glu Tyr Ala Val Gly Ile Tyr	904
140 145 150 155	
gac aac gcc atg gtc ccc cag atg tgt ggg aac ctc act ccc tcg gca Asp Asn Ala Met Val Pro Gln Met Cys Gly Asn Leu Thr Pro Ser Ala	952
160 165 170	
cac tgc atc aat gtc aga gct tcc aga gac tgc gca agc att tct tca His Cys Ile Asn Val Arg Ala Ser Arg Asp Cys Ala Ser Ile Ser Ser	1000
175 180 185	
gag gat tcg cat gat tat gtc aat gtc ccc aca gca gaa gag att gct	1048

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Glu Asp Ser His Asp Tyr Val Asn Val Pro Thr Ala Glu Glu Ile Ala
 190 195 200

gag act cta gct tct acc aaa agc cct tcc aga aat ctc ttt gtt ctt Glu Thr Leu Ala Ser Thr Lys Ser Pro Ser Arg Asn Leu Phe Val Leu 205 210 215	1096
ccc agt acc cag aag ctg gag ttt act gag gaa aga gat gag ggc tgt Pro Ser Thr Gln Lys Leu Glu Phe Thr Glu Glu Arg Asp Glu Gly Cys 220 225 230 235	1144
gga gat gct ggt gac tgc acc agt ttg tat tct cca gga gct gag gac Gly Asp Ala Gly Asp Cys Thr Ser Leu Tyr Ser Pro Gly Ala Glu Asp 240 245 250	1192
agt gat tca ctc agc aat gga gaa ggt tct tct cag atc tca aat gac Ser Asp Ser Leu Ser Asn Gly Glu Gly Ser Ser Gln Ile Ser Asn Asp 255 260 265	1240
tat gtc aac atg aca ggg ttg gat ctc agt gcc atc cag gaa agg cag Tyr Val Asn Met Thr Gly Leu Asp Leu Ser Ala Ile Gln Glu Arg Gln 270 275 280	1288
ctc tgg gtg gct ttt cag tgc tgc aga gac tat gaa aat gtt cca gca Leu Trp Val Ala Phe Gln Cys Cys Arg Asp Tyr Glu Asn Val Pro Ala 285 290 295	1336
gca gat ccc agt gga agc cag cag cag gct gag aaa gat gtg cca tcc Ala Asp Pro Ser Gly Ser Gln Gln Ala Glu Lys Asp Val Pro Ser 300 305 310 315	1384
tca aac ata ggt cat gtc gag gac aag aca gat gat ccc ggg acc cat Ser Asn Ile Gly His Val Glu Asp Lys Thr Asp Asp Pro Gly Thr His 320 325 330	1432
gtc caa tgt gtc aaa agg aca ttc ctt gct tca ggg gat tat gca gac Val Gln Cys Val Lys Arg Thr Phe Leu Ala Ser Gly Asp Tyr Ala Asp 335 340 345	1480
ttt cag cca ttc aca cag agt gag gac agt cag atg aaa cat aga gaa Phe Gln Pro Phe Thr Gln Ser Glu Asp Ser Gln Met Lys His Arg Glu 350 355 360	1528
gag atg tca aat gag gac tcc agt gac tat gaa aat gtg cta act gcc Glu Met Ser Asn Glu Asp Ser Ser Asp Tyr Glu Asn Val Leu Thr Ala 365 370 375	1576
aag tta gga ggc agg gac tct gag cag ggg cct ggc act cag ctc ctt Lys Leu Gly Gly Arg Asp Ser Glu Gln Gly Pro Gly Thr Gln Leu Leu 380 385 390 395	1624
cct gat gaa tga agacccaggt acccagccat aaagccacat tgagtgtct Pro Asp Glu	1676
atccccatagg attgactact gcagagtcta gtgcagaccc gtgatcacct tagtgcttca	1736
gtggattcac tggtagatt aaaaagaggc tgagatgagc agtgaactaa gaggccacac	1796
aaaagcagag gtttggaaat tccagaaggg aattcttctc aagcagagtg tggttatctc	1856
ctgtaccagc ctaagaatgt ttgctgaaac tgcttcctag aactgtgaag aaagcaggaa	1916

HYS-67 Sequence Listing

agtatgtcac	agtagtctaa	gattattacc	ttcattaata	ccaacaggct	gcaaaggcaag	1976
agtatagatt	attgtataat	ccagtcagag	gtcaaaagga	aggaagaagt	tggagtggag	2036
tggggtgtggc	aatttccatt	ttaaagagtg	taggc			2071

<210> 4
<211> 398
<212> PRT
<213> Homo sapiens
<400> 4

Met	Asp	Gly	Val	Thr	Pro	Thr	Leu	Ser	Thr	Ile	Arg	Gly	Arg	Thr	Leu
1				5				10				15			

Glu	Ser	Ser	Thr	Leu	His	Val	Thr	Pro	Arg	Ser	Leu	Asp	Arg	Asn	Lys
				20			25				30				

Asp	Gln	Ile	Thr	Asn	Ile	Phe	Ser	Gly	Phe	Ala	Gly	Leu	Leu	Ala	Ile
				35		40				45					

Leu	Leu	Val	Val	Ala	Val	Phe	Cys	Ile	Leu	Trp	Asn	Trp	Asn	Lys	Arg
				50		55		55		60					

Lys	Lys	Arg	Gln	Val	Pro	Tyr	Leu	Arg	Val	Thr	Val	Met	Pro	Leu	Leu
				65		70		70		75		80			

Thr	Leu	Pro	Gln	Thr	Arg	Gln	Arg	Ala	Lys	Asn	Ile	Tyr	Asp	Ile	Leu
				85		90		90		95					

Pro	Trp	Arg	Gln	Glu	Asp	Leu	Gly	Arg	His	Glu	Ser	Arg	Ser	Met	Arg
				100			105		105		110				

Ile	Phe	Ser	Thr	Glu	Ser	Leu	Leu	Ser	Arg	Asn	Ser	Glu	Ser	Pro	Glu
				115		120		120		125					

His	Val	Pro	Ser	Gln	Ala	Gly	Asn	Ala	Phe	Gln	Glu	His	Thr	Ala	His
				130		135		135		140					

Ile	His	Ala	Thr	Glu	Tyr	Ala	Val	Gly	Ile	Tyr	Asp	Asn	Ala	Met	Val
				145		150		150		155			160		

Pro	Gln	Met	Cys	Gly	Asn	Leu	Thr	Pro	Ser	Ala	His	Cys	Ile	Asn	Val
				165			170		170		175				

Arg	Ala	Ser	Arg	Asp	Cys	Ala	Ser	Ile	Ser	Ser	Glu	Asp	Ser	His	Asp
				180		185		185		190					

Tyr	Val	Asn	Val	Pro	Thr	Ala	Glu	Glu	Ile	Ala	Glu	Thr	Leu	Ala	Ser

195

HYS-67 Sequence Listing
200 205

Thr Lys Ser Pro Ser Arg Asn Leu Phe Val Leu Pro Ser Thr Gln Lys
210 215 220

Leu Glu Phe Thr Glu Glu Arg Asp Glu Gly Cys Gly Asp Ala Gly Asp
225 230 235 240

Cys Thr Ser Leu Tyr Ser Pro Gly Ala Glu Asp Ser Asp Ser Leu Ser
245 250 255

Asn Gly Glu Gly Ser Ser Gln Ile Ser Asn Asp Tyr Val Asn Met Thr
260 265 270

Gly Leu Asp Leu Ser Ala Ile Gln Glu Arg Gln Leu Trp Val Ala Phe
275 280 285

Gln Cys Cys Arg Asp Tyr Glu Asn Val Pro Ala Ala Asp Pro Ser Gly
290 295 300

Ser Gln Gln Gln Ala Glu Lys Asp Val Pro Ser Ser Asn Ile Gly His
305 310 315 320

Val Glu Asp Lys Thr Asp Asp Pro Gly Thr His Val Gln Cys Val Lys
325 330 335

Arg Thr Phe Leu Ala Ser Gly Asp Tyr Ala Asp Phe Gln Pro Phe Thr
340 345 350

Gln Ser Glu Asp Ser Gln Met Lys His Arg Glu Glu Met Ser Asn Glu
355 360 , 365

Asp Ser Ser Asp Tyr Glu Asn Val Leu Thr Ala Lys Leu Gly Gly Arg
370 375 380

Asp Ser Glu Gln Gly Pro Gly Thr Gln Leu Leu Pro Asp Glu
385 390 395

<210> 5
<211> 1197
<212> DNA
<213> *Homo sapiens*

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<400> 5
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ctgcatgtga ctccccgcag cctggacaga aataaaagacc agatcaccaa catctttcc 120
gggtttgcgg gactcctcgc catcctcctg gtcgttgcgg ttttctgcat cttgtggaaat 180

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HYS-67 Sequence Listing

tggaaaataaac	ggaagaagcg	acaagttcct	taccccgag	ttaccgtcat	gcccttgctg	240
actttgcac	aaaccagaca	aagagccaaa	aatattttag	acatcttgcc	ttggcgacag	300
gaagacctgg	ggagacatga	gtcgaggagt	atgcgcattt	tcagtactga	gaggcctcctc	360
tccagaaatt	ctgagagccc	ggagcatgtg	ccctcccaag	caggcaatgc	cttccaggag	420
catacagccc	acatccatgc	cacagagtac	gcgggtggta	tctatgacaa	cgccatggtc	480
ccccagatgt	gtggaaacct	cactccctcg	gcacactgca	tcaatgtcag	agcttccaga	540
gactgcgcaa	gcatttcttc	agaggattcg	catgattatg	tcaatgtccc	cacagcagaa	600
gagattgctg	agactctagc	ttctacccaaa	agcccttcca	gaaatcttt	tgttcttccc	660
agtacccaga	agctggagtt	tactgagga	agagatgagg	gctgtggaga	tgctggtgac	720
tgcaccagtt	tgtattctcc	aggagctgag	gacagtgatt	cactcagcaa	tggagaaggt	780
tcttctcaga	tctcaaatga	ctatgtcaac	atgacagggt	tggatctcag	tgccatccag	840
gaaaggcagc	tctgggtggc	ttttcagtc	tgcagagact	atgaaaatgt	tccagcagca	900
gatcccagtg	gaagccagca	gcaggctgag	aaagatgtgc	catcctcaaa	cataggtcat	960
gtcgaggaca	agacagatga	tcccgggacc	catgtccat	gtgtcaaaag	gacattcctt	1020
gcttcagggg	attatgcaga	ctttcagccca	ttcacacaga	gtgaggacag	tcagatgaaa	1080
catagagaag	agatgtcaaa	tgaggactcc	agtactatg	aaaatgtct	aactgccaag	1140
ttaggaggca	gggactctga	gcaggggcct	ggcactcagc	tccttcctga	tgaatga	1197

<210> 6

<211> 30

<212> PRT

<213> Homo sapiens

<400> 6

Met Asp Gly Val Thr Pro Thr Leu Ser Thr Ile Arg Gly Arg Thr Leu
 1 5 10 15

Glu Ser Ser Thr Leu His Val Thr Pro Arg Ser Leu Asp Arg
 20 25 30

<210> 7

<211> 368

<212> PRT

<213> Homo sapiens

<400> 7

Asn Lys Asp Gln Ile Thr Asn Ile Phe Ser Gly Phe Ala Gly Leu Leu
 1 5 10 15

Ala Ile Leu Leu Val Val Ala Val Phe Cys Ile Leu Trp Asn Trp Asn
 20 25 30

HYS-67 Sequence Listing

Lys Arg Lys Lys Arg Gln Val Pro Tyr Leu Arg Val Thr Val Met Pro
 35 40 45

Leu Leu Thr Leu Pro Gln Thr Arg Gln Arg Ala Lys Asn Ile Tyr Asp
 50 55 60

Ile Leu Pro Trp Arg Gln Glu Asp Leu Gly Arg His Glu Ser Arg Ser
 65 70 75 80

Met Arg Ile Phe Ser Thr Glu Ser Leu Leu Ser Arg Asn Ser Glu Ser
 85 90 95

Pro Glu His Val Pro Ser Gln Ala Gly Asn Ala Phe Gln Glu His Thr
 100 105 110

Ala His Ile His Ala Thr Glu Tyr Ala Val Gly Ile Tyr Asp Asn Ala
 115 120 125

Met Val Pro Gln Met Cys Gly Asn Leu Thr Pro Ser Ala His Cys Ile
 130 135 140

Asn Val Arg Ala Ser Arg Asp Cys Ala Ser Ile Ser Ser Glu Asp Ser
 145 150 155 160

His Asp Tyr Val Asn Val Pro Thr Ala Glu Glu Ile Ala Glu Thr Leu
 165 170 175

Ala Ser Thr Lys Ser Pro Ser Arg Asn Leu Phe Val Leu Pro Ser Thr
 180 185 190

Gln Lys Leu Glu Phe Thr Glu Glu Arg Asp Glu Gly Cys Gly Asp Ala
 195 200 205

Gly Asp Cys Thr Ser Leu Tyr Ser Pro Gly Ala Glu Asp Ser Asp Ser
 210 215 220

Leu Ser Asn Gly Glu Gly Ser Ser Gln Ile Ser Asn Asp Tyr Val Asn
 225 230 235 240

Met Thr Gly Leu Asp Leu Ser Ala Ile Gln Glu Arg Gln Leu Trp Val
 245 250 255

Ala Phe Gln Cys Cys Arg Asp Tyr Glu Asn Val Pro Ala Ala Asp Pro
 260 265 270

Ser Gly Ser Gln Gln Gln Ala Glu Lys Asp Val Pro Ser Ser Asn Ile
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HYS-67 Sequence Listing
280 285

Gly His Val Glu Asp Lys Thr Asp Asp Pro Gly Thr His Val Gln Cys
290 295 300

Val Lys Arg Thr Phe Leu Ala Ser Gly Asp Tyr Ala Asp Phe Gln Pro
305 310 315 320

Phe Thr Gln Ser Glu Asp Ser Gln Met Lys His Arg Glu Glu Met Ser
325 330 335

Asn Glu Asp Ser Ser Asp Tyr Glu Asn Val Leu Thr Ala Lys Leu Gly
340 345 350

Gly Arg Asp Ser Glu Gln Gly Pro Gly Thr Gln Leu Leu Pro Asp Glu
355 360 365

<210> 8

<211> 21

<212> PRT

<213> Homo sapiens

<400> 8

Asn Lys Asp Gln Ile Thr Asn Ile Phe Ser Gly Phe Ala Gly Leu Leu
1 5 10 15

Ala Ile Leu Leu Val
20

<210> 9

<211> 347

<212> PRT

<213> Homo sapiens

<400> 9

Val Ala Val Phe Cys Ile Leu Trp Asn Trp Asn Lys Arg Lys Lys Arg
1 5 10 15

Gln Val Pro Tyr Leu Arg Val Thr Val Met Pro Leu Leu Thr Leu Pro
20 25 30

Gln Thr Arg Gln Arg Ala Lys Asn Ile Tyr Asp Ile Leu Pro Trp Arg
35 40 45

Gln Glu Asp Leu Gly Arg His Glu Ser Arg Ser Met Arg Ile Phe Ser
50 55 60

Thr Glu Ser Leu Leu Ser Arg Asn Ser Glu Ser Pro Glu His Val Pro
65 70 75 80

HYS-67 Sequence Listing

Ser Gln Ala Gly Asn Ala Phe Gln Glu His Thr Ala His Ile His Ala
 85 90 95

Thr Glu Tyr Ala Val Gly Ile Tyr Asp Asn Ala Met Val Pro Gln Met
 100 105 110

Cys Gly Asn Leu Thr Pro Ser Ala His Cys Ile Asn Val Arg Ala Ser
 115 120 125

Arg Asp Cys Ala Ser Ile Ser Ser Glu Asp Ser His Asp Tyr Val Asn
 130 135 140

Val Pro Thr Ala Glu Glu Ile Ala Glu Thr Leu Ala Ser Thr Lys Ser
 145 150 155 160

Pro Ser Arg Asn Leu Phe Val Leu Pro Ser Thr Gln Lys Leu Glu Phe
 165 170 175

Thr Glu Glu Arg Asp Glu Gly Cys Gly Asp Ala Gly Asp Cys Thr Ser
 180 185 190

Leu Tyr Ser Pro Gly Ala Glu Asp Ser Asp Ser Leu Ser Asn Gly Glu
 195 200 205

Gly Ser Ser Gln Ile Ser Asn Asp Tyr Val Asn Met Thr Gly Leu Asp
 210 215 220

Leu Ser Ala Ile Gln Glu Arg Gln Leu Trp Val Ala Phe Gln Cys Cys
 225 230 235 240

Arg Asp Tyr Glu Asn Val Pro Ala Ala Asp Pro Ser Gly Ser Gln Gln
 245 250 255

Gln Ala Glu Lys Asp Val Pro Ser Ser Asn Ile Gly His Val Glu Asp
 260 265 270

Lys Thr Asp Asp Pro Gly Thr His Val Gln Cys Val Lys Arg Thr Phe
 275 280 285

Leu Ala Ser Gly Asp Tyr Ala Asp Phe Gln Pro Phe Thr Gln Ser Glu
 290 295 300

Asp Ser Gln Met Lys His Arg Glu Glu Met Ser Asn Glu Asp Ser Ser
 305 310 315 320

Asp Tyr Glu Asn Val Leu Thr Ala Lys Leu Gly Gly Arg Asp Ser Glu
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HYS-67 Sequence Listing

330

335

Gln Gly Pro Gly Thr Gln Leu Leu Pro Asp Glu
340 345